JOURNAL OF AGRICULTURAL RESEARCH

VOL. 53 WASHINGTON, D. C., NOVEMBER 1, 1936 NO. 9

HISTOLOGICAL AND CYTOLOGICAL CHANGES IN SUGAR-BEET SEEDLINGS AFFECTED WITH CURLY TOP[1]

By ERNST ARTSCHWAGER, *pathologist*, and RUTH C. STARRETT, *assistant cytologist*, *Division of Sugar Plant Investigations, Bureau of Plant Industry, United States Department of Agriculture*[2]

INTRODUCTION

Virus diseases, because of their weakening effect on the organism, are responsible for many stunted plants with greatly impaired reproductive capacity. The developmental disturbances are local or systemic, most easily noted in young, growing tissue, and practically absent from mature organs. During the early stages there are cytoplasmic and nuclear changes and, in the aerial parts, disturbances in the chlorophyll apparatus.

Changed nuclear forms have been observed in connection with many virus diseases, notably mosaic, but their significance has been variously evaluated. Thus, Goldstein (*12*)[3] finds the nuclei of mosaic plants hardly changed, whereas Schander and his coworkers (*16*) consider mosaic a nuclear disease entirely. A changed cytoplasmic structure, caused by extreme vacuolization and accompanying proteolysis, has also been considered an important symptom in young cells (*6*). Last but not least, the X-bodies, first discovered in mosaic plants by Iwanowski (*13*), raised high hopes and started a deluge of investigations into their nature. Once thought by some to be the cause of virus diseases, they are now mostly relegated to the position of some obscure metabolic product of the virus-infected cell.

Curly top of sugar beets (*Beta vulgaris* L.) belongs to a group of virus diseases of major economic importance, but, as in the case of all virus diseases, little is known about its nature. To be sure, much information has recently been gained from the work of Esau (*8, 9, 10, 11*) and Bennett (*4*). Thanks to their studies, we have a better understanding of the minute structure of diseased and normal phloem as well as the location and movement of the virus. Little is known as yet about the early cytological responses following infection, and it was with this in mind that the studies recorded in this paper were undertaken.

Investigations on the seedstalk of curly top beets were carried on for several seasons, but subsequent studies on diseased roots have yielded more valuable knowledge concerning cellular responses to virus invasion. The seedling root possesses a large amount of phloem, while the simultaneous activity of the supernumerary cambium provides a wealth of relatively young cells for the virus to act upon. Practically all of these immature cambium derivatives mature

[1] Received for publication Feb. 19, 1936; issued Nov. 1, 1936.

[2] The writers wish to thank L. W. Sharp, Cornell University; L. O. Kunkel, Rockefeller Institute for Medical Research; and members of the staff of the Division of Sugar Plant Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, for critical reading of the manuscript. They are indebted also to Eubanks Carsner and N. J. Giddings, senior pathologists; C. W. Bennett, pathologist; and C. F. Lackey, assistant pathologist, Division of Sugar Plant Investigations, for furnishing most of the material used in this study.

[3] Reference is made by number (italic) to Literature Cited, p. 656.

into phloem tissue and are open to virus invasion; interzonal parenchyma cells, being far less subject to disturbance by abnormal stimuli, are not differentiated until later. There are also few complications arising from the effect of secondary factors, such as disturbed chlorophyll apparatus, phloem exudate channels, and insect injuries.

Shortly after these studies were completed there appeared a paper by Esau (*11*), part of which, dealing with degeneration of the primary root, is an elaboration of her own preliminary investigations (*8*) and those of Rawlins (*15*). While there is some duplication in these studies and those recorded in the present paper, emphasis, on the whole, is put on widely different topics which make the studies complementary to each other rather than competitive.

MATERIALS AND METHODS

Most of the material for study was obtained through the cooperation of the field station of the United States Department of Agriculture at Riverside, Calif., and consisted of beet seedlings of strain 2769, very susceptible to curly top, and of the resistant strain 286. The plants were grown in pots in the greenhouse; to obtain diseased plants, virus-carrying leafhoppers were caged upon individual leaves and were removed several days later. The material was killed in several fixing fluids, and the usual methods of dehydrating and embedding in paraffin were employed.

The stain most successfully employed was Flemming's triple stain, used according to the following modified schedule: [4]

(1) Slides, with paraffin removed, in 50-percent alcohol.
(2) Stain in safranine 1 hour. (The stain is prepared by mixing equal parts of a 1-percent solution of water-soluble and alcohol-soluble safranines.)
(3) Transfer to water.
(4) Dip into acidulated 70-percent alcohol.
(5) Wash for a brief time in 70-percent alcohol.
(6) Dip into 85-percent alcohol.
(7) Dip into 95-percent alcohol.
(8) Transfer to absolute alcohol.
(9) Transfer to a mixture consisting of one-third absolute alcohol and two-thirds xylol; change twice.
(10) Stain in crystal violet 5 seconds or longer. (Prepare stain by mixing 2 cc of saturated crystal violet in clove oil with 1 cc of absolute alcohol and 50 cc of xylol.)
(11) Transfer to xylol; change four times.
(12) Stain in orange G on slide; follow staining reaction under microscope and interrupt staining at proper stage. (Stain is prepared by making a saturated solution of the powder in clove oil, filtering, and adding a few drops of xylol. Only the best orange G, preferably Grübler's, should be used to obtain uniformly good results.)
(13) Transfer to xylol; change several times.
(14) Mount in balsam.

The Feulgen chromatin reaction as given by Lee (*14*) was used in a somewhat modified form according to the following schedule:

(1) Slides, with paraffin removed, in water.
(2) Treat with normal hydrochloric acid at 60° C. for 12 minutes.
(3) Dip in water.
(4) Stain in colorless B fuchsin for 3 to 4 hours. (The stain is prepared as follows: Dissolve 1 g of basic fuchsin in 200 cc of hot water; cool to 50° C. and filter; cool down to 25° and add 1 g of potassium metabisulphite. Keep solution in the dark in a tightly stoppered bottle for at least 24 hours before using.)
(5) Transfer to a freshly prepared solution of: Water, 200 cc; normal hydrochloric acid, 10 cc; potassium metabisulphite, 1 g. Keep slides in this solution for 6 minutes.

[4] Recommended by D. M. Weller, of the University of Hawaii.

(6) Wash in running water 10 minutes.
(7) Transfer successively to 60-, 80-, 95-, and 100-percent alcohol.
(8) Transfer to xylol.
(9) Mount in balsam.

In the Feulgen reaction, the hot hydrochloric acid decomposes the thymonucleic acid contained in the chromatin. The aldehyde that is liberated forms with the leucobase of basic fuchsin an addition compound which is colored a vivid cherry red.

The material used for the Feulgen reaction was killed in a concentrated aqueous solution of corrosive sublimate to which 2 percent of acetic acid was added.

All photographs were taken on Wratten M plates with B–58 and E–22 filters used singly or in combination. The drawings[5] are mostly based on photomicrographs.

STRUCTURE OF NORMAL AND DISEASED TAPROOT

Since a detailed description of structure and development of beet roots is given elsewhere (*1*), it will suffice here to recall only the salient features. Microscopically the young beet seedling shows three regions—root, hypocotyl, and cotyledons. The junction between hypocotyl and root is indicated by an abrupt tapering of the axis and the appearance of lateral rootlets. The center of the root is occupied by a thin strand of vascular tissue, which is enclosed by a cortex and bound peripherally by a single-layered epidermis. The central cylinder of root and lower hypocotyl shows a diarch protoxylem plate with alternating phloem groups, a single-layered pericycle, and a band of parenchyma between xylem and phloem. The innermost layer of these parenchyma cells gives rise to the primary cambium, which as such develops xylem and phloem in the normal manner. Before primary growth is completed, there arises in the tissue between endodermis and primary phloem the first secondary cambium. It is derived either directly from cells of the pericycle and primary phloem parenchyma or from their descendants. The second supernumerary cambium is formed from the outer daughter cells of the first cambium initial, and additional cambiums are derived in a similar manner. In a longitudinal section of a beet root, the cells of the secondary cambiums and their derivatives have a characteristically tiered arrangement. They are very uniform, of similar size, and, when mature, make up the sieve tubes, companion cells, and parenchyma of the secondary phloem.

The phloem parenchyma cells are larger than the sieve tubes and have straight or slightly inclined end walls. They retain their nuclei and cytoplasm and may contain leucoplasts and even starch. The companion cells also possess nuclei but are usually shorter and always narrower than the phloem parenchyma cells. The sieve tubes lack nuclei and appear empty. They have well-developed terminal sieve plates, sieve-tube plastids, and, when young, variously shaped slime bodies such as those described by Esau (*10*) for the sieve tubes of the leaf.

As compared with the normal structure just described, the picture of a radial section through a root affected by curly top differs at first glance by showing a disturbance of the typically tiered arrangement

[5] All the drawings were prepared by Mrs. Eugenia Artschwager.

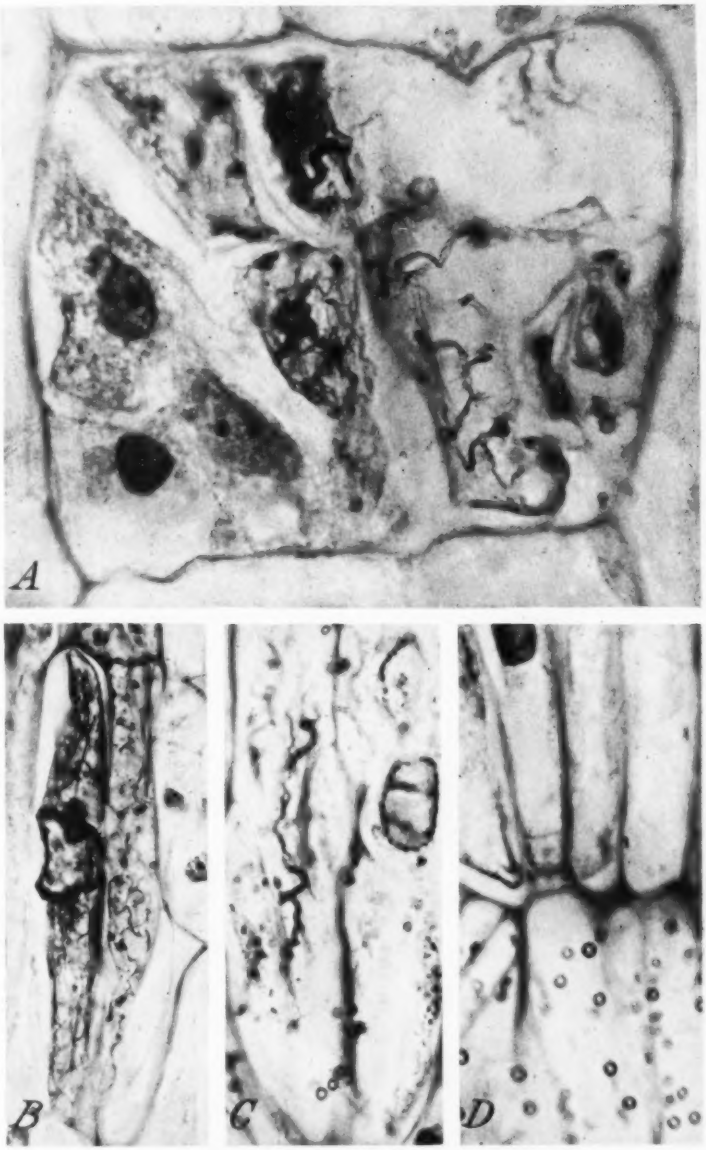
of the cells caused by the interpolation of hypertrophied and diseased cells among the normal ones. Some of these cells have enlarged uniformly, while in others the longitudinal and transverse diameters increased unequally, giving rise to greatly elongated or irregular cubical cells. Most of these hypertrophied elements are phloem parenchyma cells. They possess nuclei, however greatly changed at times, and almost all of them have peripheral cytoplasm. For the most part they are empty but may contain as transitory accompaniments abnormal amounts of leucoplasts, crystal sand, and inclusion bodies.

Scattered among the elements of the diseased secondary phloem are certain cells or cell groups which a closer study reveals to be sieve-tubelike elements. They are axially elongated, like the surrounding cells, and their end walls are for the most part straight. Occasionally the cells are pointed or quite irregular. These aberrant cell forms must be produced as a result of direct or indirect stimulation from the curly top virus, since they are wanting in normal beet tissue. Characteristic of these sieve-tubelike cells is the abundance of large sieve-tube plastids and slime bodies (pl. 1, *A–D*) and the lack of nuclei and terminal sieve plates. Withal, the tissue resembles in both origin and structure the hyperplastic cell groups recently described and illustrated by Esau (*9*) for the diseased phloem of young beet leaves. The homologous nature of these pseudosieve tubes in roots and aerial organs is confirmed by a study of the effect of curly top virus on the internal structure of young leaves and of the seedstalk of flowering beets. Plate 2 gives the sequence of development of these pseudosieve tubes in the midribs of seedling leaves. The walls of the potential hyperplastic cells are not only thickened and chemically changed, as indicated by the staining reaction, but this thickening had taken place before there was any recognizable pathological disturbance in either nucleus or cytoplasm. Details for the formation of the hyperplastic tissue are given in Esau's paper, and only such observations are here recorded as seem necessary for the general understanding of the early cytological phenomena associated with the disease.

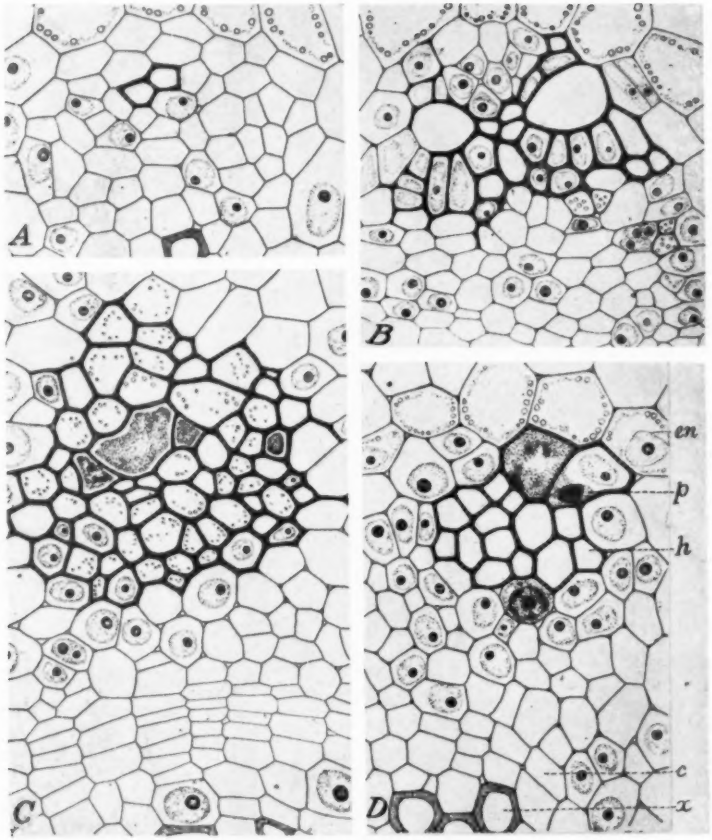
STRUCTURE OF NORMAL AND DISEASED LATERAL ROOTLETS

The lateral roots of the beet are fibrous and covered for a considerable distance with root hairs. They arise endogenously in the pericycle of the young taproot, mostly opposite the two protoxylem points, and thus appear externally in two opposite longitudinal rows. Structurally the lateral roots differ from the taproot in the very limited amount of secondary growth, the absence of supernumerary cambiums, and the occurrence among them of triarch and tetrarch forms besides the traditional diarch ones.

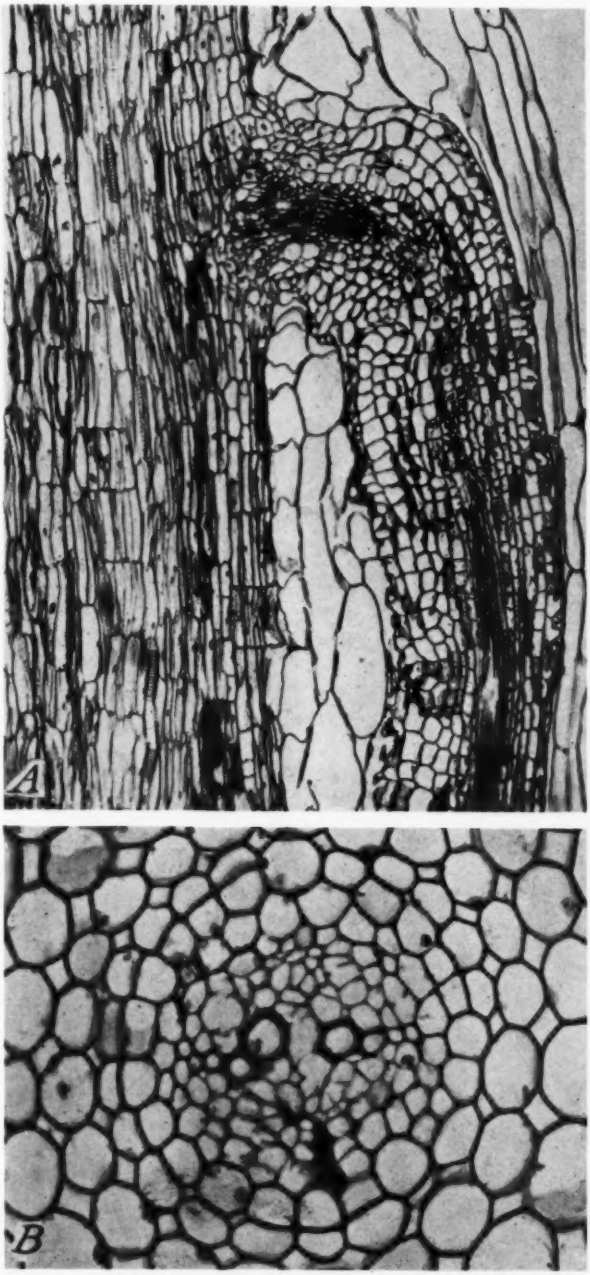
Although the number of laterals is normally large, there is a further increase in beets affected with curly top. The rootlets appear here very crowded, so much so that often two laterals arise from the same initial. As active organs of absorption, however, they have only a limited existence. The increase in the number of lateral rootlets in diseased plants may be considered a physiological response whereby new organs of absorption are formed, replacing those prematurely dead.



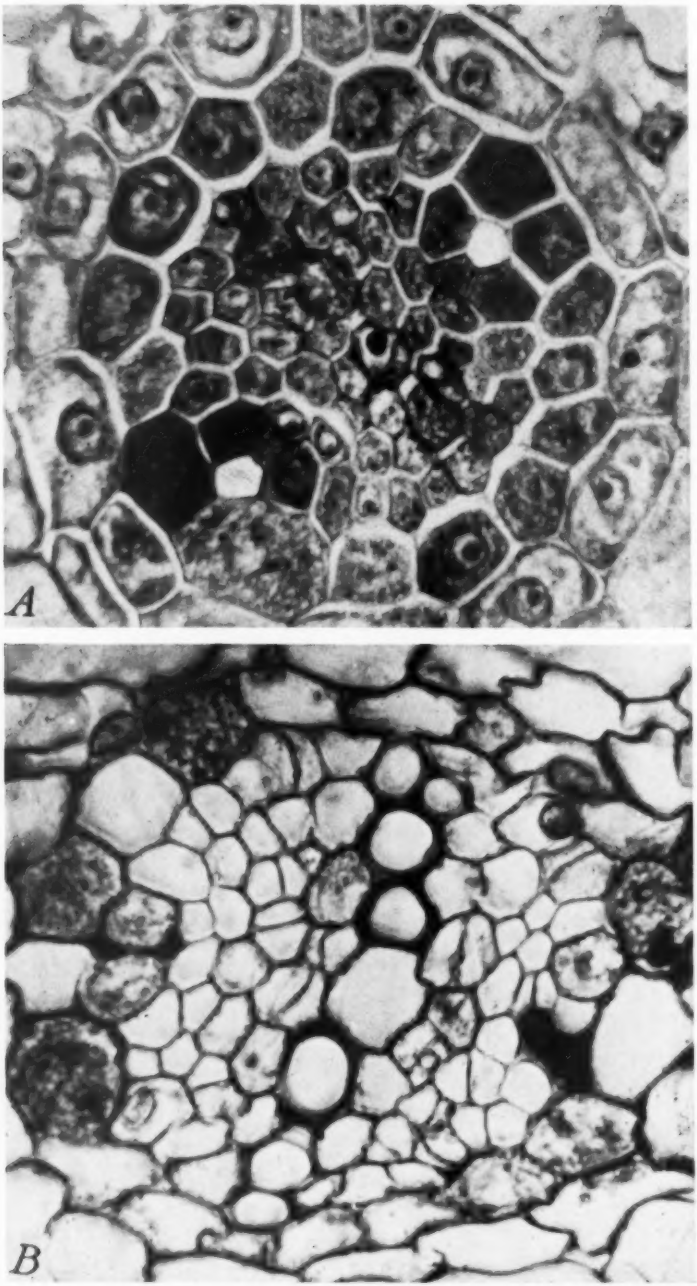
A, Young irregular hyperplastic cells from taproot of seedling beet, showing numerous slime bodies and sieve-tube plastids. Cells at left still possess nuclei. × 1,300. *B*, Narrow hyperplastic sieve-tubelike cells with numerous small slime bodies. × 710. *C*, Slime bodies and sieve-tube plastids. At right, large cleavage nucleus. × 900. *D*, Hyperplastic sieve-tubelike cells with numerous large sieve-tube plastids but without terminal sieve plates. × 1,300.



A, Cross section of midrib of embryonic leaf with two sieve tubes and one protoxylem element. The sieve tubes are separated from the endodermis by two rows of pericyclic cells. *B*, Cross section of midrib of leaf somewhat older than the one illustrated in *A*. One pericyclic cell has greatly enlarged; the potential hyperplastic tissue is indicated by the great thickness of the walls of the cells. *C*, Cross section of midrib of a still older leaf. The hyperplastic tissue is quite massive; most of the cells contain numerous sieve-tube plastids. *D*, Cross section of a lateral bundle from leaf illustrated in *C*. Two of the pericyclic cells are enlarged and their content has degenerated; the hyperplastic tissue is extensive. *en*, Endodermis; *p*, pericycle; *h*, hyperplastic tissue; *c*, cambium; *x*, xylem. The position of the endodermis in *A*, *B*, and *D* is indicated by its content. All × 748.



A, Longitudinal section of taproot and lateral rootlet. The lateral rootlet leads vertically downward through the cortex before emerging. × 95. *B*, Transverse section of a lateral rootlet with some of the cells diseased. × 810.



A, Transverse section through tip of lateral rootlet. Notice the two sieve tubes devoid of content; also enlargement in the adjacent pericyclic cells. × 1,400. *B*, Transverse section of diseased lateral rootlet. The pericyclic cells are greatly enlarged and the nuclei hypertrophied and in the process of degeneration. × 1,400.

The peculiar behavior on the part of some of the laterals in breaking through the cortex is of interest in this connection. Instead of pursuing a normal horizontal or somewhat oblique course, they continue vertically downward in the tissue of the cortex before finally breaking through the epidermis. The downward course of such a lateral is illustrated in plate 3, *A;* while *B* of the same figure gives the anatomical structure of such a rootlet in cross section. The cortex of a young rootlet is composed of only a few rows of large cells, which make a relatively broad ring around the miniature stele. The pericycle is one cell wide and abuts directly on phloem and protoxylem. The phloem appears in two oblong groups in juxtaposition. It is surrounded, except for the region of the pericycle, by more or less undifferentiated procambium. The latter is to all purposes identical with phloem parenchyma and companion cells, and only the two protophloem sieve tubes (pl. 4, *A*) are readily recognized as such.

The lateral rootlets become infected through the taproot. Since their structure is relatively simple, it is easier to trace the effect of the curly top virus here than in the complicated structure of the main root. The most striking changes, as seen in plate 4, *B*, are exhibited by the cells of the pericycle. These cells are greatly enlarged and undergo subsequent degenerative changes as the disease increases in severity. The primary sieve tubes become occluded with a red-staining gummy substance or they appear completely collapsed with only the walls showing. Degeneration of the primary sieve tubes, however, is a normal phenomenon, and the process in diseased roots may only be hastened and otherwise slightly changed. Depending on the severity of the disease, other tissues, including cortex and endodermis, become involved; and in the extreme form of the disease the rootlet becomes reduced to a necrotic mass of cells in which the original structure is hardly discernible.

DEVELOPMENT OF CALLUS AND PSEUDOCALLUS

Diseased taproots of young beet seedlings often show peculiar beadlike protuberances on the walls of the sieve tubes and sieve-tube-like elements described above. These swellings are found on both terminal and lateral walls. They vary greatly in number and size. Occasionally only terminal swellings are found, but more often lateral ones are also present. The terminal swellings are almost always massive, while those along the lateral walls are small or medium, and, since they normally appear on opposite sides of the same wall, they impart to the latter a peculiar beaded appearance (pls. 5, *A;* 6, *F*). Where sieve tubes and pseudosieve tubes border on pericyclic or phloem parenchyma cells, swellings are found only along the walls of the sieve tubes and pseudosieve tubes (pl. 6, *C*). Large lateral swellings are commonly found near the end of the cell, but may occupy any place along the lateral wall.

The swellings at first glance suggested callus formations, but subsequent studies showed them to be different in origin and composition. Normal callus (pl. 6, *A*, *B*) appears rather early in the primary sieve tubes of the root. Like any normal callus, it first shows as a uniform deposit over one side of the sieve plate, plugging the pores of the latter. The callus deposit, quite thin at first, becomes massive (*B*), effectively sealing the terminal wall of the now functionless sieve tube.

Compared to the normal callus, the wartlike swellings just described differ in being equally massive on either side of the sieve plate, in developing in places where normal callus is not formed, and, finally, in not giving the characteristic microchemical callus reaction. For the sake of convenience the calluslike wall swellings will be referred to as "pseudocallus."

The terminal pseudocallus appears as an abnormal wall swelling which is faintly divided by the differently staining middle lamella (pl. 6, *H*) into two more or less equal parts. The outer part of the swelling is always much darker than the inner substance, but old swellings may be uniformly dark throughout (*G*). The developing terminal callus often stains brightly with orange G, and the matrix of the thickened part is foamy or spongy (*E*) instead of having the consistency of a homogeneous gel. Another frequently observed form of terminal callus is shown in *D*. It looks like a large blister compressed into the lumen of the rather narrow element; the swelling is divided by a thin, indistinct middle lamella. The body of the swelling appears as a homogeneous substance bounded peripherally by a dark crust. This peculiar callus formation may be observed in all types of material, but it is relatively conspicuous in resistant varieties, since in these abnormalities of other types are rare.

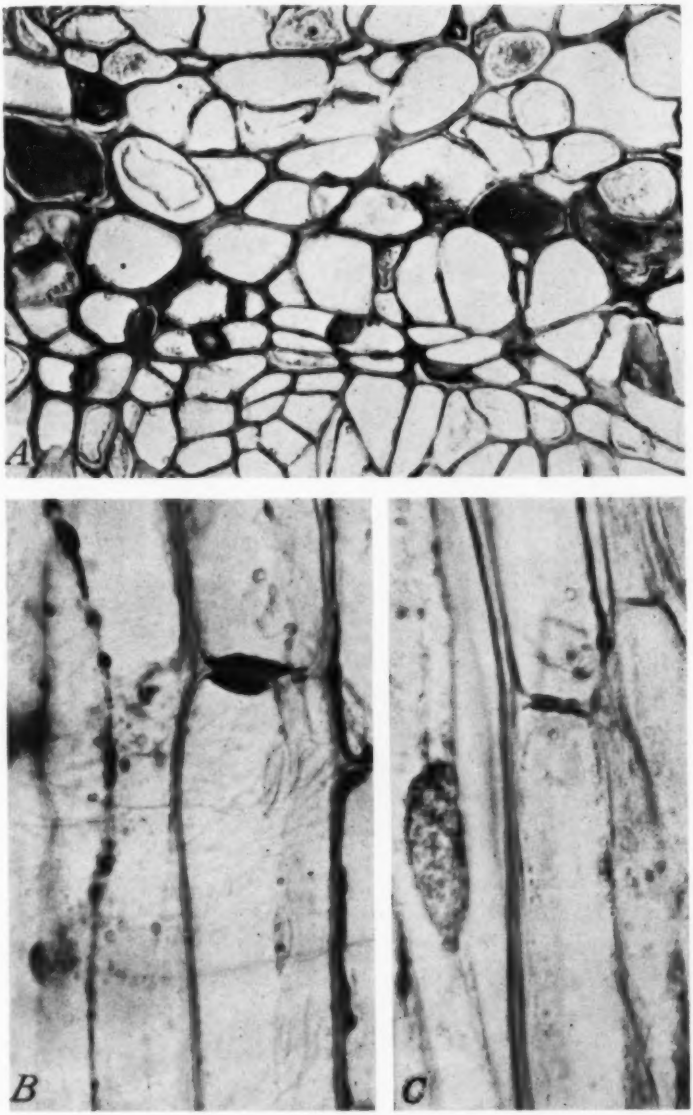
The lateral pseudocallus is structurally quite uniform, the outer shell taking a deeper stain than the central zone. It may be found in any stage of development, with small and massive forms occurring side by side on the same cell wall.

Whether developed terminally or along the side walls, the pseudocallus is initiated as a small discoloration of the outer lamella of the primary wall as seen in plate 5, *A–C*. The discolorations in the terminal wall soon become confluent, but the lateral swellings remain localized or may unite incompletely if development is extensive. The discolored areas gradually thicken, assuming varied forms, as shown in plates 5 and 6. Their ontogeny is controlled by the metabolism of the host cell, whose life in turn is affected by many factors such as age, relative position within the tissue, degree of stimulation (by the virus or its products), and food supply.

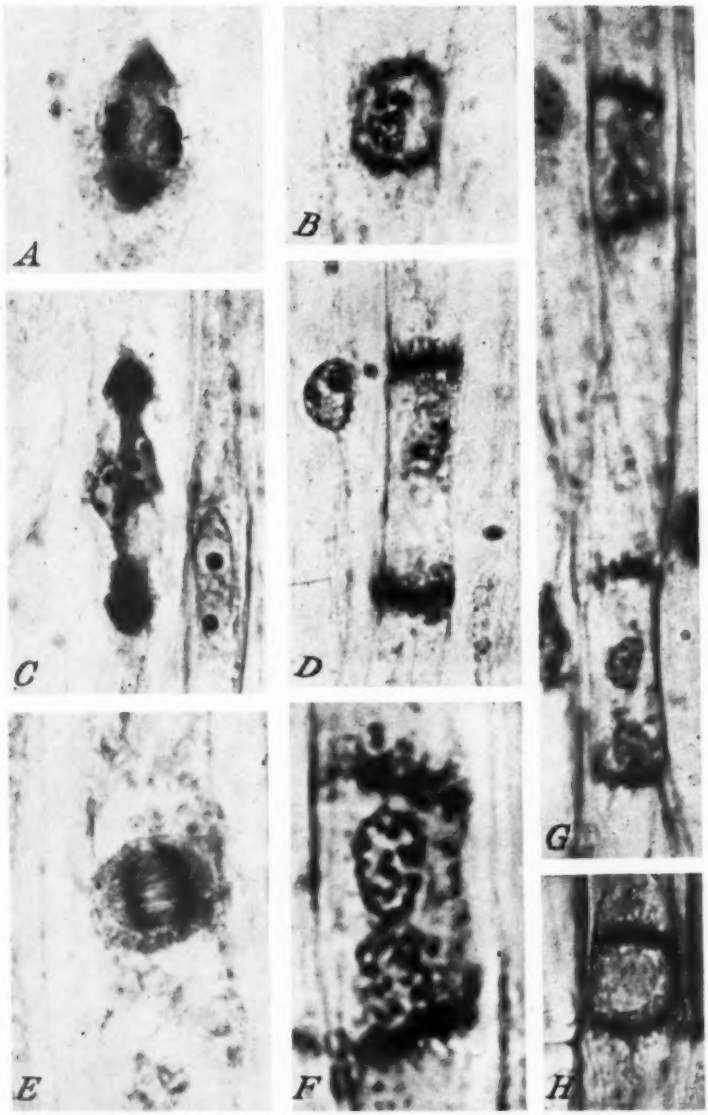
The formation of pseudocallus is one of the earliest reactions on the part of the host to curly top infection. It is observed before the advent of abnormal cell division and becomes conspicuous after the development of hyperplastic sieve-tubelike tissue is in progress. It is found frequently in resistant plants where internal symptoms are often altogether wanting. The swellings are naturally more pronounced in the taproot of seedlings than in the lateral rootlets, since the latter develop relatively little secondary tissue and rarely ever hyperplastic cells.

NORMAL AND ABNORMAL LONGITUDINAL DIVISION

The axially elongated cells of the sugar-beet rootlet, notably those of the pericycle and cambium, divide longitudinally after a pattern described by Bailey (*2*) for the cambium cells of the white pine. In beets affected with the curly top disease, where cells of abnormal dimensions are not uncommon, this peculiar type of division is found not only in axially elongated cells but also in relatively short elements; in fact, in cells which may be all but isodiametric and where the usual type of cell-wall formation should be the rule.



A, Transverse section of phloem of first supernumerary ring of diseased seedling showing pseudocallus in all stages of development. × 940. *B*, Advanced stages of pseudocallus formation. × 1,360. *C*, Initial stages of terminal and lateral pseudocallus formation. × 1,360.



A, Normal sieve-tube callus in young sieve tube. *B*, Older callus in mature sieve tube. *C*, Terminal and lateral pseudocallus. Notice its absence from the walls of the phloem parenchyma cells. *D*, Massive terminal pseudocallus. *E*, Spongy terminal pseudocallus. *F*, Beaded appearance of lateral pseudocallus. *G*, Old lateral and terminal pseudocallus, very homogeneous in nature and staining almost black. *H*, Greatly enlarged old terminal callus. All × 1,450.

During telophase of such longitudinal division, the spindle substance widens in the equatorial region to form a barrel-shaped phragmoplast, which then extends laterally until it comes in contact with the end walls of the cell. During this process the spindle substance soon disappears near the nucleus but continues at the margin of the growing cell plate by the addition of accessory fibers that make up the two secondary phragmoplasts. The cell plate, according to present-day concept (*17, p. 174*), appears as a liquid film at the equator at the close of mitosis; the deposition of salts and other matter in the film transforms it into the middle lamella. The secondary phragmoplasts are located halfway between the tangential walls of the cell and are usually equidistant from the daughter nuclei (pl. 7, *A*, *C*). This symmetrical arrangement is not always observed with this type of division, for the distance of each of the daughter nuclei frequently shows variation in relation to the approximate center of the protoplast. The phragmoplasts, as described by Bailey for the white pine, have a wedge-shaped outline, bluntly convex in front and tapering to a point along the rear of the cell plate. In sugar beets they have the form of a blunt cone (*A*), with the flat side in the direction of the nucleus. From this flat side the fibers taper rather gradually along the cell plate and finally disappear.

There is little variation in the size of the phragmoplast, except that cells which have a large primary spindle tend to have larger phragmoplasts, and these are commonly, though not always, found in large cells. The fibers of the phragmoplast closely resemble those of the normal spindle. Of course, they are much shorter than the normal fibers and extend only partly across the cell lumen. The terminal portions of the cell plate, with the two phragmoplasts, travel to their respective ends of the cell, leaving a fully formed cell plate behind them. The latter is extended until the cell is completely divided into halves, each of which contains one of the daughter nuclei.

Binucleated cells are occasionally found in healthy beet roots, but they are of common occurrence in plants affected with the curly top disease. According to Beer and Arber (*3*), binucleated cells are frequently found in growing tissue of higher plants. These investigators also found certain peculiar features connected with the mitosis and behavior of the associated protoplasm, a behavior which Bailey (*2*) later encountered and illustrated in his study of division in the cambium cells of the white pine. It appears that the daughter nuclei in telophase were found to be enclosed in a hollow sphere of dense protoplasm, the appearance suggesting a cell within a cell. Bailey strongly suspected the phenomenon referred to by Beer and Arber to be a phase of cytokinesis not unlike that illustrated by him.

Since the root tissue of beets offers an unusual opportunity for the study of this peculiar and probably little known phenomenon, it might not be out of place to give a brief account of it in this paper.

The initiation of cell division leading to the binucleated condition follows the normal course. There is nothing unusual in the appearance of the metaphase spindle or in the succeeding stages except that in late anaphase the cytoplasm concentrates rimlike about the karyokinetic figure (pl. 7, *E*). With the disappearance of the spindle fibers and the reconstruction of the daughter nuclei, this rim of kinoplasm becomes quite distinct, forming, to use the words of Bailey (*2*), a halo around the daughter nuclei (*B*, *H*). In cells of

large diameter, this rim of kinoplasm widens, and when it intersects the radial walls of the cell it becomes more or less angular. There is no wall formation in progress between the daughter nuclei. The kinoplasm now disappears from the side walls of the rectangular rim, leaving two granular masses which extend from one radial wall to the other (*D*, *G*). The granular masses of fibrillae, comparable to the phragmoplasts of the normal longitudinal-division figures, now move in opposite directions (*D*) until they eventually reach the ends of the protoplast. The office of the phragmoplasts in the formation of the new wall in cells dividing longitudinally is clear, even though we may not comprehend the mechanism by which this is accomplished; it would be futile, however, to attempt a reasonable explanation for the rather complicated method of division in cells where cell-wall formation between the daughter nuclei is never realized even in the initial stages.

The course of reconstruction of the daughter nuclei resulting from this type of division is usually belated. The chromatin often remains in the form of chromosomes (pl. 7, *F*) long after the phragmoplasts or their equivalents have receded from the daughter nuclei and are well on their way, moving in opposite directions toward the ends of the cell. Nuclei formed in this peculiar manner occasionally possess a larger chromosome complex; even tetraploidy has been observed in them.

REACTION OF THE NUCLEUS TO CURLY TOP INFECTION

CHANGES IN SIZE AND FORM

Giant nuclei are of common occurrence in beets affected by curly top. The cells in which they are found are also enlarged, though nuclear hypertrophy may be observed in cells of normal size, in which case the nucleus fills the greater part of the cell lumen.

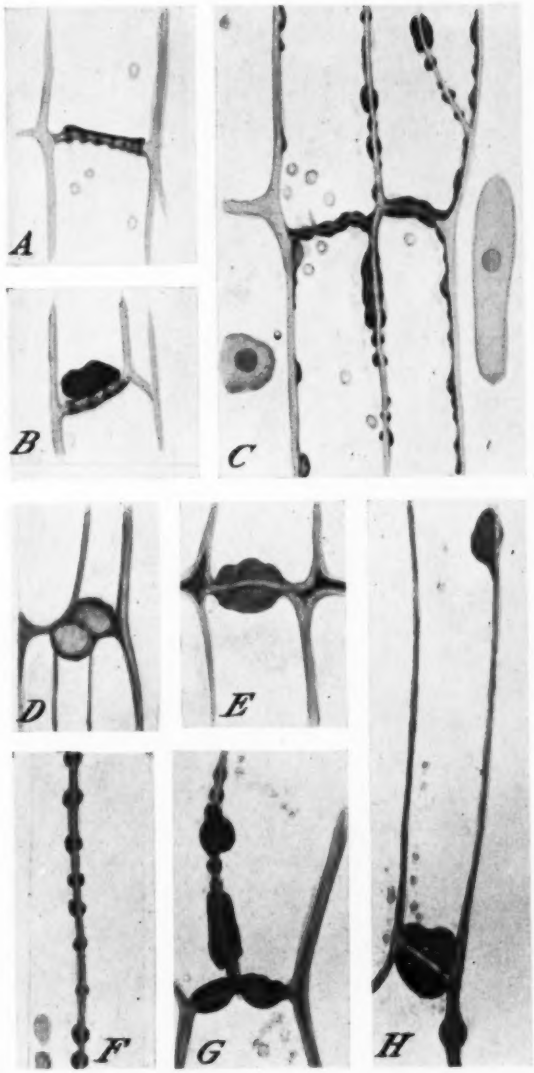
The enlarged nuclei are mostly round. Other nuclear forms are largely bound up with the shape of the cells; thus, in greatly elongated cells, the nuclei are also exceptionally long. On the other hand, spherical nuclei are also observed in elongated cells (pl. 8, *A*) and are found almost without exception in all irregular cell forms.

Cells with enlarged nuclei belong morphologically to the pericycle or the phloem parenchyma, depending on the age and type of root. Thus, in young laterals the cells of the pericycle are hypertrophied, whereas in taproots, where secondary growth has already become pronounced, the nuclei of the phloem parenchyma are those mostly affected.

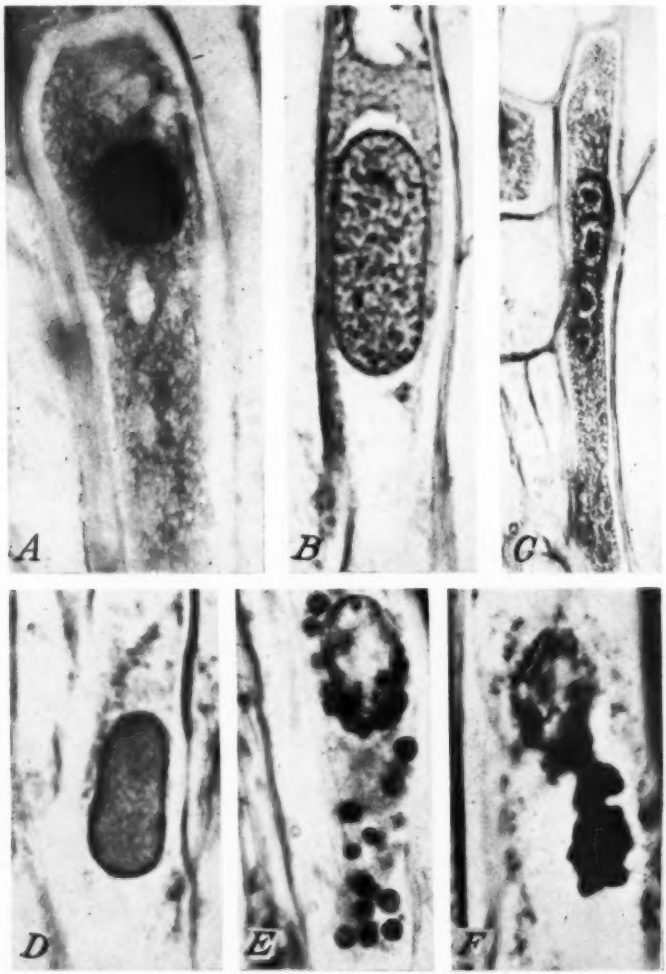
Increase in nuclear size may occasionally be brought about by nuclear fusion, the process being often facilitated through the multinucleated condition of the affected cells. Such compound nuclei, however, are readily recognized, even though the separate nuclear membranes have disappeared. (See pl. 11, *J*.)

Amoeboid nuclei are not uncommon. They are often observed in cells of endodermis and cortex but rarely ever in the cells of the vascular tissue. Edson (7) described and illustrated such irregular nuclear forms in beet seedlings affected with *Phoma betae*, but did not indicate in what tissue they occurred.

In the process of hypertrophy, nuclei may become irregular, notched, cleft, or deeply segmented. An extreme type of this group



A, Phragmoplasts and early cell-wall formation in longitudinally dividing cell. × 1,450. *B*, Early stage in binucleated cell formation. × 1,450. *C*, Later stage in normal longitudinal division. × 1,400. *D*, Later stage in binucleated cell formation. × 1,450. *E*, Late anaphase preceding stage illustrated in *B*. × 1,350. *F*, Stage similar to *D*, except that the nuclei have the chromatin still in the form of contracted chromosomes. × 2,000. *G*, Two abnormal cell divisions in one cell row. × 1,000. *H*, Stage similar to *B*; the phragmoplasts form a circle, but the nuclei are not seen in this particular focus. × 1,350.



A, Enlarged phloem parenchyma cell of taproot with dense vacuolate cytoplasm and large spherical nucleus undergoing chromatolysis. × 1,200. *B*, Hypertrophied nucleus with coarse flaky chromatin. × 1,400. *C*, Narrow phloem parenchyma cell with greatly elongated nucleus; the chromat in forms four pseudonuclei, each surrounded by a light halo. × 1,200. *D*, Hypertrophied nucleus with dense nuclear membrane and finely dispersed chromatin. Nucleoli are absent. × 1,400. *E*, Phloem parenchyma cell with large nucleus practically devoid of chromatin. There are many leucoplasts with starch. × 1,200. *F*, Greatly enlarged phloem parenchyma cell. The nucleus has only a little chromatin. Large inclusion body resembling chromatin extrusion in close proximity to the nucleus. × 1,500.

is the "cleavage nucleus", so called because of its resemblance to the mulberry fruit or the blastula stage of an embryo. Nuclei of this type are shown in plates 9 to 11. They are most commonly derived from the spherical or oval nucleus, but occasionally are related to the greatly elongated nuclear type. There appear to be two distinct classes of cleavage nuclei. To the first class belong those in which the cleavage furrows progress inwardly from the periphery. In their early developmental stages these differ from normal nuclei only in the somewhat scalloped appearance of the wall (pl. 11, *G*). As the invaginations deepen, the nucleus becomes divided into a number of segments which usually adhere to one another, so that the nucleus continues to retain its original size and shape. The segments into which the nucleus divides are usually of unequal size, the central segments being larger than the peripheral ones (pls. 9, *B*, *C*, *E*, *H*; 10, *I*). Occasionally segmentation is very symmetrical, so that the divided nucleus has a more or less honeycombed appearance (pls. 9, *F*; 11, *I*). In the nuclei of the second class, cleavage is entirely internal (pls. 8, *C*; 9, *G*; 10, *C*), so that in the initial stages such nuclei give the appearance of vacuolization, owing to poor fixation. As the cleavage progresses, the membranes of the individual segments are quite firm and distinct. The original nuclear membrane persists or may disintegrate, leaving the segmented inner region in the form of a mass of loosely adhering bubbles. Most of the nuclei as described and illustrated here are not distinct types, but rather transitory forms marking definite steps in the degenerative process.

CHROMATIN

The normal nucleus, according to the present-day concept (*17*), is composed of karyolymph and a reticulum of chromatic threads. The former composes the bulk of the nucleus and is in either the sol or the gel state. A division of the reticulum into chromatin and linin cannot easily be made objectively, since the existence of two distinct morphological substances is attributed by some workers to differences in degree of condensation of a single reticular substance; likewise, a division of the chromatin into oxychromatin and basichromatin may be related to the physical condition of the chromatin. Recent protoplasmic studies point more and more to the fact that nuclear substances are not chemically defined nucleoproteins but substances of changing composition, formed from a positive and a negative colloid.

Knowledge of the colloidal structure of the nuclear substances and their metabolism is still very insecure, and a distinction between normal and pathological is too often made subjectively. Ultramicroscopic studies show that even slight injuries suffice to cause flocculation of the chromatin (*18, p. 434*); chemical changes may cause irreversible gel formation, and frequently the close proximity of a parasite is sufficient to bring about such changes.

In beets infected with curly top the effect of the virus on the chromatin, as judged by its morphological structure, is manifold. The chromatin may appear flaky, coarsely granular (pls. 8, *B*; 11, *A*), finely divided, or entirely devoid of structure. In nuclei of the last-named type, the pronounced staining reaction shows the chromatin in a state of dissolution. Nuclei with finely divided chromatin (pls. 8, *D*; 9, *B*, *E*) frequently show clumping in localized regions; while in

some, distinct chromocenterlike masses are discernible (pl. 9, *A*). In certain nuclei the chromatin has contracted into a central granular core, which is separated from the nuclear membrane by a more or less colorless zone. Stained according to Feulgen, only the center gives a positive chromatin reaction, while the peripheral zone remains hyaline. In other nuclei of this type the central chromatin core is broken up into a number of irregular lumps, which are either located at the periphery or are scattered throughout the interior of the nucleus. Finally, nuclei are found which contain only a few bright-staining granules of chromatin or inorganic protein crystalloids.

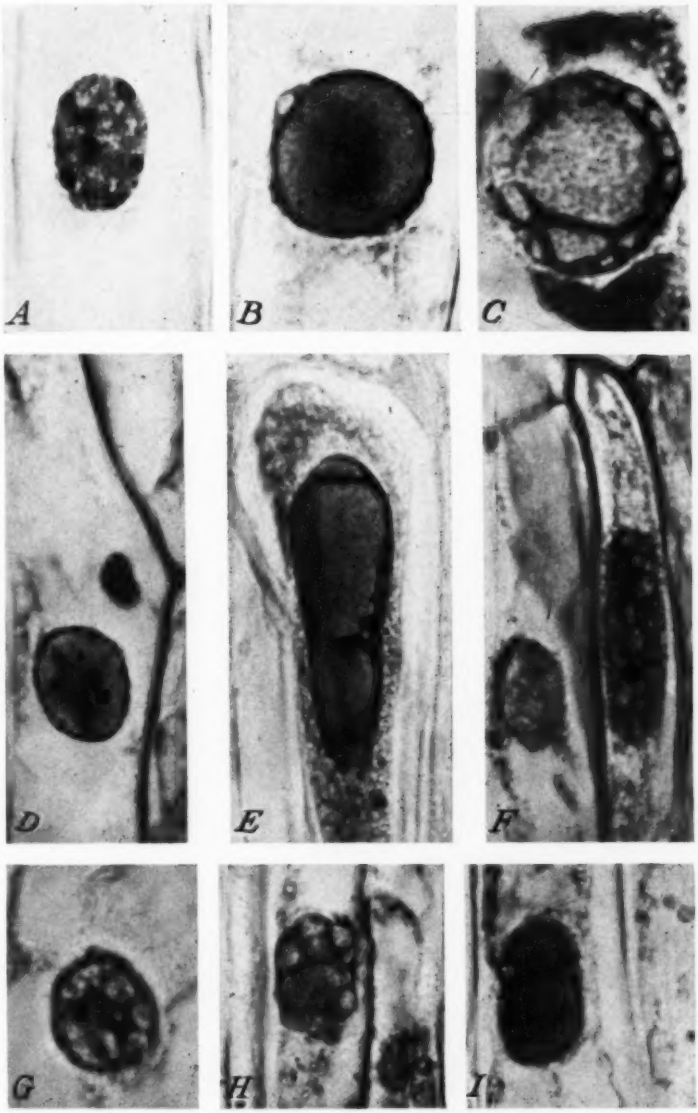
The chromatin of newly infected pericyclic nuclei with distinct hypertrophy is at first coarsely granular; later stages show regional clumping; and finally, under the influence of nuclease or of some other factor, the chromatin dissolves and may pass through the weakened and ruptured nuclear membrane (pl. 10, *G*) into the cytoplasm.

NUCLEOLUS

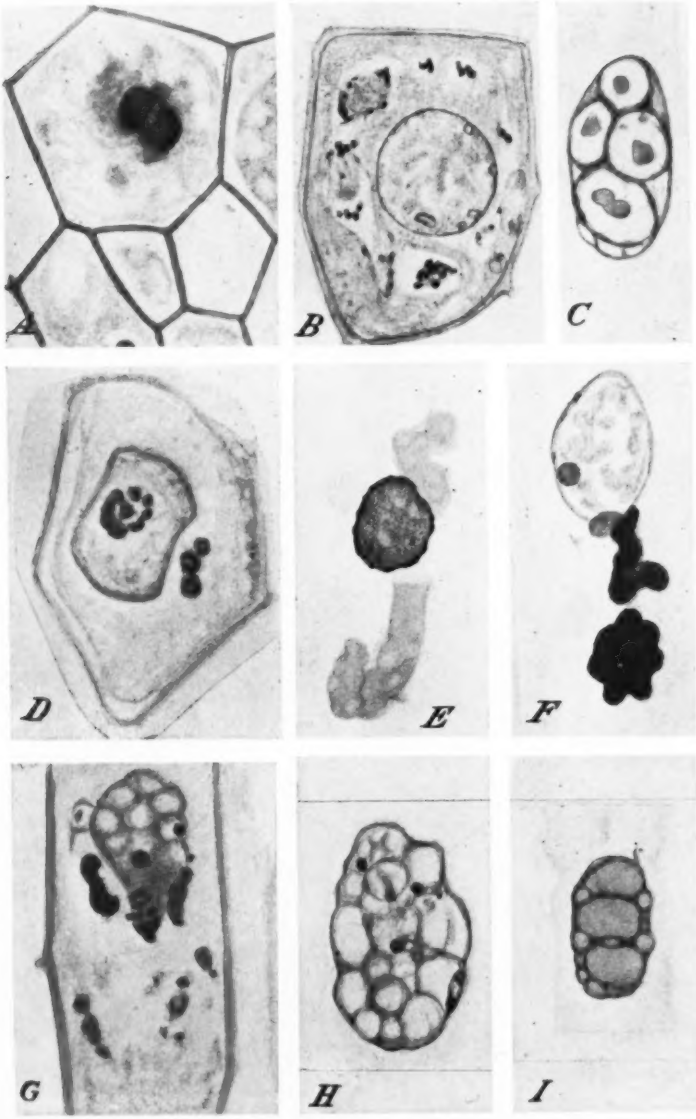
All nuclei of the root cells of sugar beet contain one or several nucleoli. The latter are usually spherical and sometimes contain one or several vacuoles. In diseased cells, the appearance of the nucleolus is quite variable and there is a certain correlation between chromatin and nucleolar behavior. One notices extreme variation in nucleolar size and number in looking at hypertrophied nuclei at random. There are nuclei, notably those with reduced chromatin content, which have a single nucleolus but of unusually large dimensions. There are others where increase in nuclear substance is brought about through the formation of accessory nucleoli varying more or less in size (pl. 11, *F*). Such numerical increase is often pronounced in greatly elongated nuclei and in those of extreme cleavage type (pl. 10, *H*). Again, there are nuclei where the nucleolus is greatly reduced in size or from which it has disappeared entirely. To this type belong mostly the hyperchromatic nuclei. In general, it may be said that hyperchromasy is associated at the beginning with increase in nucleolar substance, which is followed by a gradual subsidence ending at times in the complete disappearance of the nucleolus.

This behavior of the nucleolus can best be studied in newly infected pericyclic cells of lateral rootlets. The nucleoli of these cells show at first a definite hypertrophy followed by fragmentation (pl. 10, *A*, *D*). The nucleolar fragments may sometimes gather at the periphery and during subsequent changes pass through the weakened or ruptured nucleolar membrane into the cytoplasm.

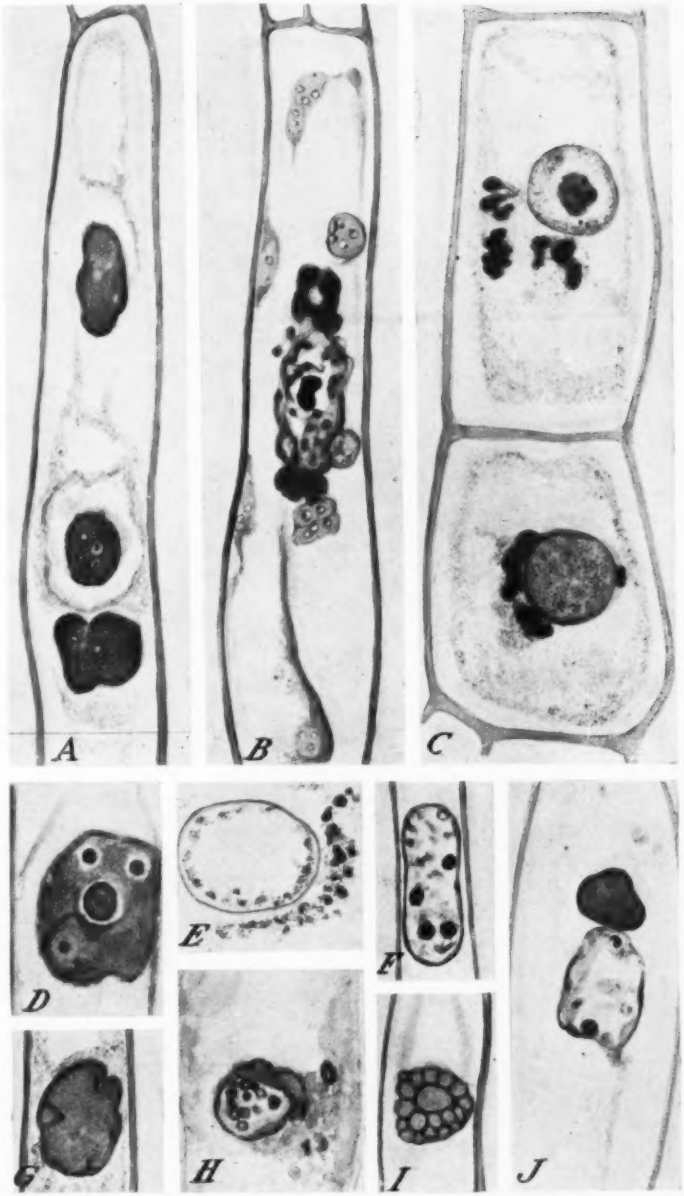
These pathological changes in the chromatin and in the nucleolar complex, as herein described, are more pronounced than those caused by other virus diseases, especially mosaic. Certain early stages in the nuclear pathology of curly top have much in common with changes observed in host nuclei of plants affected with mycorrhizal fungi or plant and animal parasites (*18, pp. 212, 240*). Such changes occur normally in the tentacle cells of *Drosera* after excitation (*18, p. 211*), but differ from pathological changes in being reversible, with the nucleus returning to normality after the subsidence of stimulation.



A, Nucleus with dense chromatin and distinct chromonemata; nuclear membrane faint. × 1,400. *B*, Greatly hypertrophied nucleus showing the beginning of peripheral cleavage. × 1,450. *C*, Nucleus similar to *B* with advanced peripheral cleavage. × 2,000. *D*, Nucleus from greatly enlarged cell; nuclear membrane thick and beaded; chromatin homogeneous, containing a few nucleolar fragments. × 1,500. *E*, Greatly hypertrophied nucleus in early stages of cleavage. × 1,450. *F*, Mulberry type of cleavage nucleus in narrow phloem parenchyma cell. × 1,450. *G*, Honeycomb type of cleavage nucleus. Segmentation is internal and does not involve the nuclear membrane. × 1,500. *H*, Extreme type of irregular cleavage. × 1,450. *I*, Similar to *H* but illustrating a different type. × 1,450.



A, Hypertrophied pericyclic cell of young lateral rootlet with empty primary sieve tube at lower right-hand corner. The nucleus of the pericyclic cell has degenerated, the nucleoli are greatly enlarged, and the nuclear membrane has disappeared. × 3,000. *B*, Hypertrophied pericyclic cell with enlarged nucleus, fragmented nucleolus and inclusion bodies; the latter are of cytoplasmic origin and resemble division stages of some chytrid parasite. × 3,000. *C*, Peculiar type of cleavage nucleus. There is slight peripheral cleavage while the center is occupied by a number of globules, each with a chromatin center. × 2,000. *D*, Hypertrophied young pericyclic cell with typical nucleolar fragmentation. × 2,000. *E*, Enlarged nucleus and inclusion body in phloem parenchyma cell stained according to Feulgen. × 1,450 *F*, Enlarged nucleus and inclusion body stained in the ordinary way. The inclusion body takes a typical chromatin stain and gives the appearance of having been formed from chromatin diffused through the nuclear membrane. × 1,450. *G*, Nuclear degeneration and nucleolar fragmentation in pericyclic cell. × 2,000. *H*, Extreme type of cleavage nucleus. Distinct nuclear membrane absent; nucleoli numerous. × 2,000. *I*, Typical cleavage nucleus; nucleoli are wanting. × 1,400.



A, Hypertrophied phloem parenchyma cell from tap root of beet seedling. The chromatin of the nucleus is contracted into a central clump while at either side of the nucleus is a large compact inclusion body. × 1,500. *B*, Similar to *A*. The nucleus has a brightly stained nucleolus; the two inclusion bodies are in contact with the nucleus. There are a number of swollen starch-bearing leucoplasts. × 1,500. *C*, Similar to *A* and *B* except that the two cells are younger. The chromatin in the nucleus of the upper cell is contracted; the inclusion bodies are in the nature of partly discrete masses. The chromatin in the nucleus of the lower cell is finely dispersed, the inclusion bodies similar to those of upper cell. × 1,400. *D*, Fusion nucleus. × 1,500. *E*, Nucleus from an old hypertrophied phloem parenchyma cell. The nucleus contains little chromatin. The mass outside the nucleus is in part amorphous precipitate from an inclusion body, in part cytoplasm. × 1,500. *F*, Somewhat hypertrophied nucleus with a number of nucleoli. × 1,500. *G*, Initial stage of peripheral cleavage. × 1,500. *H*, Nucleolar fragmentation with nucleus in last stage of degeneration. × 1,500. *I*, Mulberry type of cleavage nucleus. × 1,500. *J*, Phloem parenchyma cell with nucleus and single compact inclusion body. × 1,550.

NUCLEAR MEMBRANE

Pathological disturbances in the nucleus frequently involve the nuclear membrane, as shown by its modified appearance and staining reaction. Such changes are not surprising if the nuclear membrane is viewed as a semipermeable layer at the nucleocytoplasmic interface, governing the transfer of substances between cytoplasm and chromatin. It is conceivable that the virus or a toxin associated with the virus, after permeating the cytoplasm, would induce, directly or indirectly, permeability changes in the nuclear membrane and subsequently initiate the far-reaching disturbances in chromatin and nucleolus described above; or the visible changes in the nuclear membrane could be considered a belated secondary phenomenon, since no effect on the structure of the membrane becomes evident until after the chromatin has been much damaged.

Changes in the structure of the nuclear membranes are progressive or regressive, and not reversible.

Progressive changes comprise a thickening of the nuclear membrane (pl. 9, *D*). The thickening is usually uniform but occasionally discontinuous, giving the nuclear wall a beaded appearance. Nuclei with membranes of either type are common to phloem parenchyma cells (pl. 8, *B*, *D*), though the beaded form is usually found in connection with chromatolytic degeneration. The thickening of the nuclear membrane is at times more apparent than real and is occasioned by the deposition of chromatin on its inner boundary. The beaded appearance of certain membranes is probably the result of unequal deposition of chromatin, as shown very strikingly in preparations stained according to Feulgen.

Regressive changes cause a gradual attenuation of the membrane, leading often to its complete disappearance. This type of degeneration is found mostly in newly affected pericyclic cells of lateral rootlets (pl. 12). Obliteration of the nuclear membrane is also observed in extreme forms of cleavage nuclei. The nuclear segments, instead of adhering, may scatter through the cytoplasm and give the cell a pseudomultinucleate appearance. A very faint nuclear membrane is occasionally observed in certain hypertrophied nuclei of phloem parenchyma cells (pl. 9, *A*), though, as a rule, the walls of such nuclei are abnormally thickened.

GENERAL PATHOLOGY

The various types of nuclear abnormalities recorded by investigators during the past decades are grouped by Tischler (*18, p. 439*) under the following headings: Karyorrhexis, pyknosis, chromatolysis, and karyocholosis. These groups are briefly characterized as follows:

(1) *Karyorrhexis, or normal old-age death.*—This is commonly observed in antipodal and tapetal nuclei, also in those of bacterial galls, and in tissues after exposure to X-rays. In such nuclei the chromatin complex condenses into clumps of varying dimensions which later fragment into smaller bodies and finally pass through the nuclear membrane into the cytoplasm, where they are absorbed.

(2) *Pyknosis.*—This is found only in relatively young cells, and the primary cause is thought to be overproduction of basic chromatin. The nucleus takes on a spiny appearance, gradually loses its turgidity, and grows smaller; while the chromatin, which at first showed a strong contraction, dissolves and diffuses through the nuclear membrane.

(3) *Chromatolysis.*—Like karyorrhexis, this is a phenomenon of old age and affects normally the nuclei of sieve tubes, vessels, and tracheids. It is characterized by the dissolution of the basic chromatin, often preceded by the flocculation of the latter. The dissolved chromatin passes through the nuclear membrane and may collect in drops on its outer boundary. If stained prior to its diffusion through the membrane, the content of such nuclei appears as a homogeneous dark mass.

(4) *Karyocholosis.*—This designates an abnormal increase in nucleolar substance, usually accompanied by a reduction in chromatin, and is caused by an inhibition of the nuclear metabolism.

The nuclei of beets infected with curly top, as previously described, do not fit exactly into any of the four groups mentioned above.

Most pronounced is the phenomenon of karyocholosis, which is always associated with some type of chromatin aberration. Thus, increase in nucleolar substance, whether brought about by the enlargement of a single nucleolus or by fragmentation and subsequent growth of the fragments, is observed in young and old nuclei, in nuclei affected with karyorrhexis or pyknosis, or in those undergoing chromatolytic dissolution.

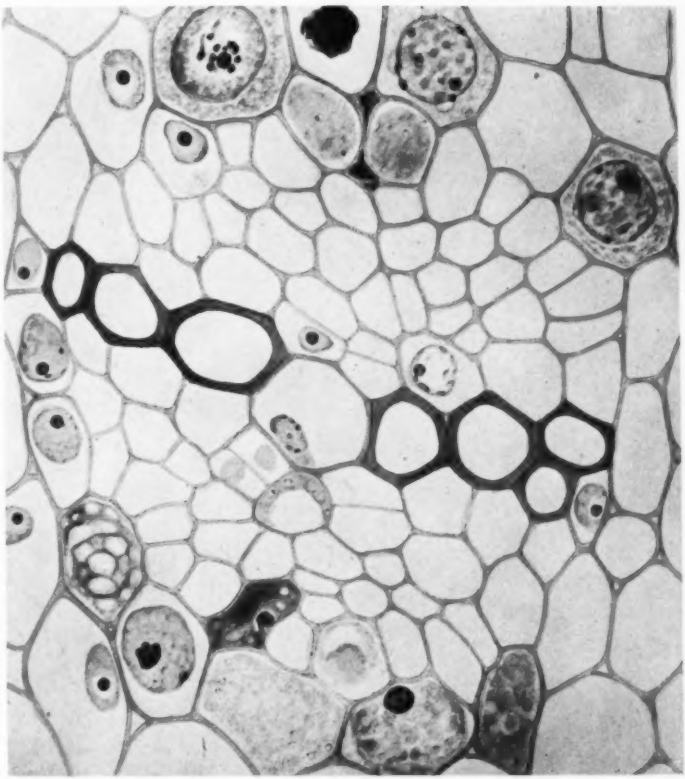
Hypertrophied nuclei of phloem parenchyma or still undifferentiated cambium derivatives of diseased taproots most often show karyorrhexis. The nuclear membrane appears thickened, and the chromatin forms irregular lumps and is contracted into a central core or finely dispersed. The presence of compact or lobed aggregates in the vicinity of the nucleus is suggestive of chromatin emission, but the negative Feulgen reaction argues strongly against their nuclear origin.

The spiny nuclear forms characteristic of the later stages of pyknotic degeneration have not been observed, though the chromatin configurations typical of the early stages of this type of nuclear disorder are seen frequently. As previously stated, pyknosis is found in relatively young cells, such as are represented by the pericycle of newly infected lateral rootlets. The nuclei of these cells show at first a pronounced overproduction of chromatin and karyocholosis. Nuclear enlargement, however, as a rule is not followed by chromatin contraction and reduction in mass, but often ends with a rupture of the nuclear membrane and the subsequent intermixing of nucleoplasm and cytoplasm.

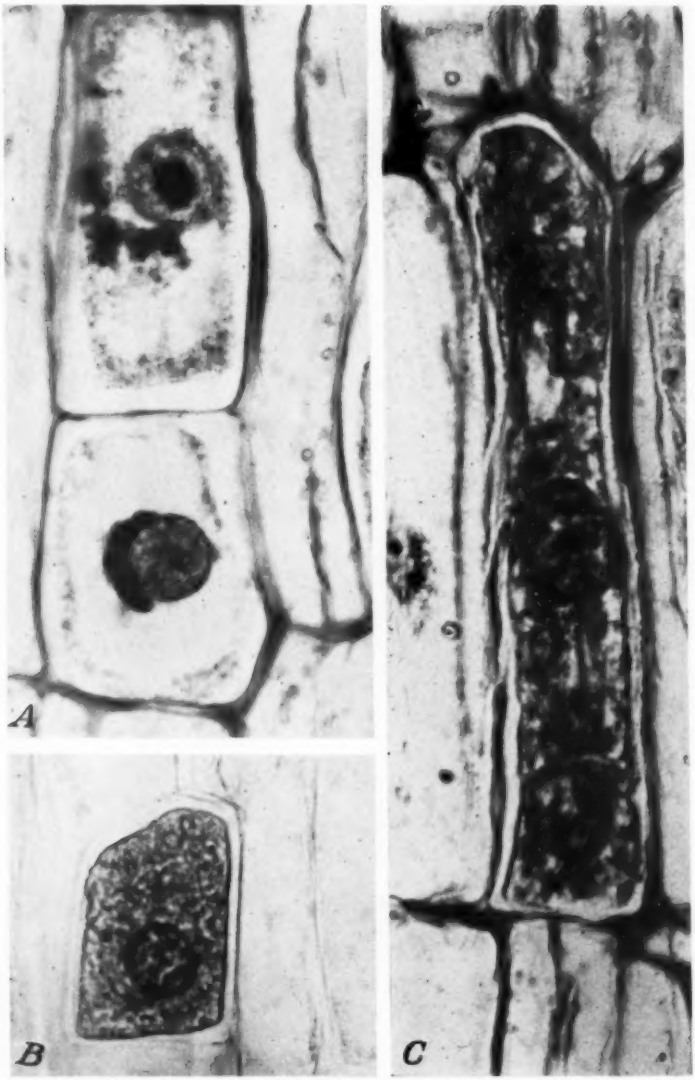
Chromatolysis as a separate symptom of degeneration is probably of rare occurrence, but it often accompanies karyorrhexis and pyknosis.

CYTOPLASMIC CHANGES

Cytoplasmic disturbances in cells affected with curly top are often less pronounced and, as a rule, less readily recognized than changes in the nucleus, although the cytoplasm comes under the influence of the parasite sooner than the chromatin. The reaction of the cytoplasm to virus infection is prompt and varied, controlled by the type of cell and the degree of its maturity. In the pericycle of young laterals which become infected through the diseased taproot, the cytoplasm of the affected cells is uniform in appearance; while in the taproot, where many of the cells have already reached a certain degree of differentiation, the pathological picture varies with each cell or group of cells. In other words, the nature and progress of pathological changes depend largely on the age at which the cell comes under the influence of the curly top virus.



Cross section of diseased lateral rootlet about 7 days after inoculation. The primary protophloem sieve tubes are completely collapsed; the pericyclic cells are greatly hypertrophied and their nuclei are in various stages of degeneration. × 1,600.



A, Two phloem parenchyma cells from a taproot of seedling, showing inclusion bodies. *B*, Broad phloem parenchyma cell with dense cytoplasm and chromatin, surrounded by empty cells. *C*, Phloem parenchyma cell from taproot seedling soon after infection, showing dense cytoplasm and chromatin. All × 1,400.

Since cytoplasmic disturbances, whether microscopically recognizable or not, should precede any nuclear disorganization, the initial stages of such disturbances are to be expected in cells where the nucleus appears still more or less normal. In cells with pronounced nuclear pathology, the diseased cytoplasm, if there is any remaining, is no longer suitable for study. Likewise, a study of the cells that become affected during the later stages of their ontogeny, when the amount of cytoplasm is greatly diminished, is of value only so far as the nucleus is concerned. Such belated disturbances are probably seldom recognized, since it appears that only relatively undifferentiated cells suffer ill effects from the virus.

The bulk of the root tissue of young beet seedlings is composed of cambium cells and young phloem tissue, part of which is in the process of differentiation. For a study of cytoplasmic changes following curly top infection, the young phloem parenchyma cells are especially instructive. The sieve tubes are of little interest, since their cytoplasm is normally scanty and soon becomes limited to a thin parietal layer. More interesting are the hyperplastic cells, those sieve-tubelike elements which play such a prominent part in the pathological picture of the phloem in the aerial organs. They seldom form a solid tissue in infected roots but appear scattered, supplanting sieve-tube and phloem parenchyma in localized regions; they are probably the result of misdirected differentiation in certain cambium cells. The cytoplasm of these cells is fibrillar rather than granular and stains more deeply than that of the surrounding cells. At an early age in their ontogeny the hyperplastic cells possess slime bodies and sieve-tube plastids which are more prominent than those found in ordinary sieve tubes and which are slow to disintegrate, being still conspicuous at a time when the slime bodies of the sieve tubes have already disappeared.

During the early stages of infection the young phloem parenchyma cells contain, as a rule, an abundance of densely staining cytoplasm. The affected cells occur singly or in groups (pl. 13, *B*, *C*), indicating a certain preference on the part of the virus or, what is likely, the ability of certain cells to overcome infection. The fixed cytoplasm is structurally finely granular (pl. 8, *C*) or fibrillar with knoblike thickenings at the intersection points of the threads. The nuclei of these cells either are still normal or are undergoing progressive modifications due to the early stimulating effect of the virus. Simultaneous with the first cytoplasmic response is a gradual enlargement of the cell as a whole. The duration of this first or stimulative phase in the degeneration of the cytoplasm is hard to predict, being influenced by the reaction of the nucleus as well as by the environment, i. e., the normal or diseased condition of the surrounding cells. The cytoplasm may continue to increase in mass, but it frequently fails to keep pace with the growth of the cell. As the disease progresses, degenerative changes in the cytoplasm become more and more evident. The picture becomes still more confusing through the appearance of inclusion bodies and increased interference on the part of the nucleus, which, as already pointed out, often undergoes chromatolytic changes resulting in emission of nucleolar fragments and possibly other nuclear material into the cytoplasm. Eventually the cytoplasm of all hypertrophied cells is reduced, often so drastically as to leave the cells practically empty. At times, however, changes are of a different nature, when

the cytoplasm and its inclusions become rapidly transformed into a homogeneous red-staining substance filling part or all of the cell lumen.

CELL INCLUSIONS

The phloem parenchyma cells of beet roots contain leucoplasts as normal cytoplasmic inclusions. Their number is small but may materially increase (pl. 8, *E*) following curly top infection. As the disease advances, the leucoplasts often degenerate, forming, together with the crumbling protoplasmic structure, those gummy cell occlusions which are among the characteristic features of the last stages in curly top degeneration.

Calcium oxalate is of common occurrence in the aerial and underground organs of the sugar beet. In the taproot, it is in the nature of crystal sand, which is limited in its distribution to a few phloem and interzonal parenchyma cells. In diseased roots, the number of calcium oxalate cells increases greatly, often so much as to impart a silvery sheen to a longitudinal section viewed under low power. The crystal-bearing cells are devoid of cytoplasm and nucleus and normally remain unstained, but occasionally they color distinctly. An explanation for this phenomenon might be sought in a possible modification of the organic substrate of the crystalline deposit, resulting in absorption of the stain.

There remains to be considered a rather important group of abnormal ergastic structures generally known as inclusion bodies.

These bodies, first noted in sugar beets by Rawlins (*15*), were recently described and illustrated by Esau (*11*), who confirmed the observations of Rawlins but modified them by putting the X- and Y-bodies into one class. The inclusion bodies, according to Esau, are first observed in young cells in close proximity to the protophloem sieve tubes. They are seen as small dark bodies, which subsequently enlarge, ranging in appearance from irregularly lobed aggregates to more or less round compact structures. The compact form is of short duration, as it soon breaks up into a conglomerate of dark globules embedded in a light-staining substrate. The disintegrating body appears to be contained within a vacuole, though no distinct membrane is discernible. The large compact bodies occur singly, in pairs, or in irregular dark clumps. Although not commonly associated with the nucleus, they are occasionally found in such close proximity to the latter as to deform it. In staining reaction and in structure, the compact bodies resemble nuclear material, but they are not of nuclear origin.

Since the investigations of Esau and those recorded in this paper are based on similar material, it is only natural that there is a certain agreement in the description of the anatomical pictures. There is, however, also a complementary value in these two records because of the difference in interpretation of apparently similar phenomena, resulting from the use of different stains, especially the Feulgen chromatin reaction, and from the subjective preference in viewpoint.

The different types of inclusion bodies may be divided roughly into two groups: Those which form irregularly lobed aggregates (pls. 8, *F*; 10, *F*; 11, *C*; 13, *A*) and those forming round compact bodies. The inclusions of the first group vary in size from isolated oval bodies to large connected or discrete masses, which at times fill a considerable part

of the cell lumen. Their relative position in the cell is indefinite and their orientation in regard to the nucleus follows no rule. The large compact bodies of the second class occur singly (pl. 11, *J*) or in pairs (pl. 11, *A*, *B*) and are then in juxtaposition with the nucleus. The inclusion bodies of the taproot may be found in any of the undifferentiated cambium derivatives which in a young seedling form the major part of the root tissue. The cells which contain them usually show signs of incipient degeneration. Such cells are usually enlarged and may contain an abundance of lininlike cytoplasm, but, as a rule, the amount of cytoplasm is greatly diminished. The nucleus is also enlarged, and the chromatin is finely granular or in irregular lumps which at times are contracted into a central core. Nucleoli are often wanting or, if present, no longer react to the safranine stain. Leucoplasts are usually present, often in large numbers, and are swollen and frequently chemically changed.

Structurally the inclusion bodies are very homogeneous, with no trace of granulation. Vacuoles are faint but relatively more prominent in Feulgen preparations (pl. 10, *E*). The staining reaction of the inclusions to Flemming's triple stain is like that of the chromatin retained in the nucleus, usually dark lavender; but in preparations stained according to Feulgen, this similarity disappears. It is at first rather difficult to locate these bodies in a Feulgen preparation, unless the condenser of the microscope is lowered; and even then their outline is quite faint

Esau's speculations concerning the ontogeny of the inclusion bodies are problematical. Although it seems safe to assert that the small rounded bodies are first seen in cells which by the appearance of cytoplasm and nuclei suggest recent infection, the larger and apparently older bodies occur in practically any type of affected cells, regardless of size and content. There is little to suggest an evolution from the discrete and loose aggregates to the large compact type. On the other hand, it appears to be true that occasionally compact inclusions break up, filling what appear to be large vacuoles with their debris. Although the inclusion bodies often appear shortly after infection and may assume prominence after the cells show the effect of cytoplasmic and nuclear degeneration, the duration of their existence is hard to determine. Since they are found just as abundantly as other types and since they are prominent in young and old cells alike, it would seem that the compact stage is not necessarily passed through rapidly. In judging the age of the inclusion bodies by the general appearance of the affected cells and their nuclei, it must be remembered that degenerative cell changes may at times be very rapid and that such changes are related to the initial infection and to the degree of maturity of the affected cell.

Judged by their differential staining pattern with Flemming's triple stain, the inclusion bodies strongly suggest a nuclear origin. Esau (*11, p. 420*) also speaks about their similarity in appearance to masses of chromosomes, and Tischler's figure (*18, p. 444, fig. 244*) could easily find a counterpart in many of the microscopic pictures from infected beet tissue. However, there is much variation in the appearance of nuclei that contain inclusions. While some of the enlarged nuclei are practically empty, others have a superabundance of chromatin. The negative Feulgen reaction speaks strongly against their chromatin nature, but it could be argued that the chromatin,

prior to emission, was so greatly altered that the Feulgen reaction was no longer positive; in that case, the chromatin retained in the nucleus should also be colored only faintly, which, however, is not the case. Occasionally, however, nuclei are associated with large inclusion bodies in which most of the chromatin retained in the nucleus stains very faintly or not at all.

It is equally difficult to link the formation of the inclusion bodies to local changes in the cytoplasm, at least in newly infected cells which are hypercytoplasmic. It is conceivable, however, that local proteolysis in the cytoplasm plays an active part in the formation of inclusions in cells where changes are so rapid that the entire content becomes metamorphosed into a multicolored mass, filling all or the greater part of the cell lumen.

Since a nuclear or cytoplasmic origin of many of the inclusion bodies is problematical, we must consider most of them byproducts, however specialized, of the pathological metabolism of the infected cells. As such, they would be homologous with similar bodies associated with mosaic and other virus diseases by numerous investigators since the days of Iwanowski.

Of more than passing interest are the inclusion bodies in affected pericyclic cells. Here the struggle between the virus or its toxin and the host cell is more swift and deadly than in the immature cambium derivatives and in the phloem parenchyma of the taproot, and the ever-changing microscopic pictures which mark the contest succeed each other quickly and are often difficult to interpret. The stimulative phase, leading to general hypertrophy, hyperchromasy, and karyocholosis, soon gives way to the destructive phase, characterized by nucleolar fragmentation and dissolution phenomena in chromatin and cytoplasm. The nucleolar fragments escape into the cytoplasm, where they are recognized by their staining reaction. However, not all of the red-staining inclusions are nucleolar in origin; some, such as those shown in plate 10, *G*, are due to local changes in the cytoplasm. Quite often these local changes that lead to the formation of pseudonucleolar inclusion bodies are so general that a major part of the cytoplasm stains more or less intensely with the safranine component of the triple stain. Often the diseased pericyclic cells contain as transitory accompaniments aggregates of chromatin and inclusion bodies, but these structures are not as clearly delimited as those described for the phloem parenchyma of the taproot.

Mention should also be made of peculiar intensely blue-staining cytoplasmic inclusions which in their shape resemble individual raphide crystals. They are of frequent occurrence in newly infected cells and are not unlike certain bodies described by Clinch (*5*) for some solanaceous plants.

DISCUSSION

The following discussion of the results of this investigation aims to establish the sequence of events by interpreting the anatomical pictures of root tissue of curly top plants and to evaluate the underlying causes for selective cellular injury through an appraisal of the normal potencies of such cells.

In lateral roots, with their limited amount of stelar tissue, the cells most easily affected are those of the pericycle, a single-layered tissue just inside the endodermis. In certain regions of the stele, pericyclic

cells abut externally and laterally on the virus-bearing primary sieve tubes (pl. 4, *A*), while centripetally to the latter is undifferentiated procambium. Since the chances for coming under the influence of the virus are equal and yet a pronounced susceptibility is shown only by the cells of the pericycle, an inquiry into its properties becomes desirable in order to help explain the differential resistance to the virus. Structurally the pericycle of roots is an unusual tissue, giving rise, as the need may be, to meristems, which form lateral roots, phellogens, and secondary cambiums, as well as adventitious roots or stems. The cells of such a tissue would naturally be very reactive, and their nuclei and cytoplasm would be most labile and responsive to stimuli even under normal conditions. In the presence of specific foreign irritants, such as a virus or toxins, one could expect far-reaching changes in the cellular metabolism that would eventually cause irreversible alterations in the nucleus and finally death. That this actually happens has been shown in the present paper.

Cell response to primary infection in the taproot does not deviate fundamentally from that in laterals, if one takes into account the differences in the structure of these two organs. The young taproot at time of infection possesses one or two supernumerary cambiums, each with several rows of immature derivatives. Because of this anomalous structure, the lateral spread of the primary infection is not uniformly centripetal or centrifugal but sporadic, depending on the distribution of the sieve tubes in the immature supernumerary rings.

The cells of the seedling taproot, which are the first to become diseased in the course of primary infection, are immature cambium derivatives, lineal descendants of the original pericycle and primary phloem parenchyma. At the time of exposure to the virus, most of these cells are little specialized, the protoplasm being somewhat less labile and more resistant to stimuli than that of the pericycle of lateral rootlets. This is evident from the following facts: The time required for abnormalities to appear is extended beyond that observed for the pericycle; the struggle between host protoplasm and virus is prolonged; the reactions are more elastic, not always terminating in early death but leading to the development of unusual cellular hypertrophies and nuclear monstrosities, causing frequently such deviations from normal development as the creation of cell types intermediate between sieve tubes and phloem parenchyma.

It appears that early primary infection in both taproot and laterals is dependent on relatively meristematic totipotent cells with labile protoplasm which reacts readily to stimuli. Such prerequisites are possessed by cells of the pericycle and the undifferentiated cambium derivatives.

The sequence of events in the cellular pathology of beets infected with curly top is normally easily followed in the case of lateral rootlets, in which the changes occur chiefly in the pericycle. But in the complicated structure of the taproot, with various types of cambium derivatives, each having its own ontogeny, analysis becomes difficult.

The first microscopically recognizable changes in the pericycle of the rootlet are noticed about 3 days after inoculation, while according to Esau (*11, p. 405*), initial disturbances in the leaf may show as early as the second day after insect feeding. The pathological pictures indicate the presence of two more or less distinct phases: An

anabolic phase, leading to general hypertrophy, and a catabolic or destructive phase, terminating with the death of the cell. The threshold of the catabolic phase finds the affected cells greatly enlarged, the nuclei hyperchromatic and the nucleoli often fragmented; the staining reaction of chromatin and cytoplasm is usually pronounced. In the course of these developments the structure of the chromatin often becomes alveolar or coarsely reticulate, the wide meshes suggesting internal cleavage (pl. 10, *G*). Destructive changes may set in while hypertrophy is still in progress and are indicated by dissolution phenomena in the nucleus and proteolysis in the cytoplasm. Simultaneously or soon afterward, nucleolar fragments and altered bits of chromatin pass through the weakened or partially destroyed nuclear membrane into the cytoplasm. Here they mingle freely with cytoplasmic fragments (pl. 10, *G*) altered by local proteolysis, and, because of similar staining reaction, soon lose their visible identity. More or less indefinite inclusion bodies assume temporary prominence in the pathological picture. They may disappear entirely or become transformed into amorphous precipitates and, when catabolic changes are very rapid, they form an integral part of the red-staining occlusions which fill the entire cell lumen.

The pathological picture of the diseased taproot is complicated because of the diverse nature of the cambium derivatives, and in analyzing the various cellular and nuclear anomalies it is essential not to confound parallelism with developmental succession.

In healthy beets, the cambium derivatives mature into sieve tubes, companion cells, and phloem parenchyma; but in diseased individuals there are, in addition, elements that are intermediate in structure between sieve tubes and phloem parenchyma. These pseudosieve tubes are absent from early primary infection but become increasingly conspicuous after the disease is well established. They are readily recognized by their numerous slime bodies and sieve-tube plastids. The accompanying cell-wall changes are very striking and have been described in detail herein, under "Pseudocallus."

The first microscopic changes in the taproot are limited to those cambium derivatives which mature into phloem parenchyma. Since their number is relatively small, moreover, and since they are less susceptible than pericyclic cells, the pathological picture of a young taproot 4 to 6 days after infection does not depart significantly from the normal structure. As a rule the cellular and nuclear changes are slow, though at times there is an almost instantaneous metamorphosis of the cell content into a gummy cell occlusion, which explains the sporadic appearance of severely diseased cells in otherwise normal tissue. Cytoplasmic and nuclear changes agree in principle with those described for the pericycle, except that the changes are less conspicuous and do not always lead to the destruction of the cell content but rather to the development of abnormal nuclear forms and transitory inclusion bodies.

The different types of nuclear anomalies have been described herein; it will suffice to call attention once more to the fact that they are not distinct forms but transition stages of certain types, of which the cleavage nucleus is the most conspicuous. The impetus for the development of the different types is probably given at the time of infection and is related to the degree of cellular susceptibility and the

quantity of the initial stimulus. This, however, does not preclude the fact that subsequent changes in the environmental conditions of the affected cell might change the normal course of nuclear pathology. Great susceptibility, coupled with maximum initial stimulation, probably results in more pronounced nuclear anomalies than does belated infection, although the actual proof would be difficult to produce. The ontogeny of a typical cleavage nucleus usually has its beginning in superficial imaginations which soon become cleavage furrows, dividing the content into asymmetrical pieces. Since cleavage nuclei are not observed until about 8 days after infection and since at the time of initial invagination the nuclei are already hypertrophied and the nucleoli often fragmented, cleavage is, in most cases, a secondary phenomenon.

The changes in the nucleocytoplasmic relationship following infection depend on initial stimulation, age, and the relation of the affected cell to its immediate environment. Whether the first effect is on the cytoplasm or on the chromatin cannot be judged objectively, since there are no known methods fine enough to serve as indicators for such early changes; however, it is undoubtedly true that even very slight changes will tend to upset the finely adjusted balance in the protoplasmic structure. Changes in the nucleus, however slight, must needs be felt in the cytoplasm; and changes in the latter will tend to further upset the normal order, until the cumulative effect of such stimulation prevents reversibility and leads to the death of the cell. The formation of aberrant cytoplasmic inclusions and the emission of nucleolar fragments and possibly of much altered chromatin are closely related to the cellular metabolism, with the time factor playing an important and varied role.

SUMMARY

Judged by its effect on the inner root structure, curly top of sugar beet is primarily a nucleocytoplasmic disorder, but its study is of interest also because of the peculiar influence of the virus on the ontogeny and structure of certain cells of the stelar tissue.

Early primary disturbances are limited to the pericycle and the immature cambium derivatives, in other words, to cells which are multipotent and possess labile protoplasm, normally very responsive to stimuli.

Under the influence of the virus, the affected cells and their nuclei hypertrophy. In this process, the nuclei may expand symmetrically, become irregular, or assume odd shapes, as exemplified by the "cleavage nucleus." Many of these abnormal nuclear forms are transitory stages of certain basic patterns, more or less predetermined at time of infection but often modified through secondary stimulation.

The changes in the affected cells are marked by two phases. These usually overlap and their relative duration appears to be dependent on the age of the cell at the time of infection, its immediate environment, and the degree of initial stimulation. During the first or anabolic phase, there is an increase in nucleolar material and chromatin, accompanied by changes in the morphological structure of the latter. The threshold of the second or catabolic phase marks the beginning of irreversible changes in the nucleus, which are often characterized by dissolution phenomena and the possible emission of

altered chromatin into the cytoplasm. Nucleolar fragments also find their way into the cytoplasm and remain there unaltered, but often lose their visible identity because of proteolytic changes in the latter.

Cytoplasmic changes usually parallel those in the nucleus during the early part of the disease; later stages are marked either by mere quantitative reduction or by disorganization due to local or general proteolysis.

There are two types of cytoplasmic inclusions. To the first group belong calcium oxalate and leucoplasts; they are normally found in healthy plants but often increase enormously in diseased roots. To the second group belong structures foreign to healthy cells. Prominent, though transitory in nature, are nucleolar fragments and possibly chromatin extrusions with the chromatin in a greatly altered state, staining only very faintly by the Feulgen method. Amorphous precipitates that show great variation in staining reaction are also very common. Their origin is not always clear but can be attributed to the disintegration of certain compact inclusion bodies and to local proteolysis of the cytoplasm.

As a result of what appears to be misdirected differentiation in the maturation of cambium derivatives, there are formed hyperplastic cells, sieve-tubelike elements with plastids and slime bodies but without sieve plates. The walls of the cells become covered with pseudocallus.

The cells of the supernumerary cambiums and their derivatives divide longitudinally after the method of cambium cells first described by Bailey (*2*) for the white pine. Incomplete longitudinal divisions, resulting in binucleate and multinucleate cells, are quite common.

LITERATURE CITED

(1) Artschwager, E.
1926. anatomy of the vegetative organs of the sugar beet. Jour. Agr. Research 33: 143–176, illus.

(2) Bailey, I. W.
1920. the cambium and its derivative tissues. III. a reconnaisance of cytological phenomena in the cambium. Amer. Jour. Bot. 7: 417–434, illus.

(3) Beer, R., and Arber, A.
1915. on the occurrence of binucleate and multinucleate cells in growing tissues. Ann. Bot. [London] 29: [597]–598.

(4) Bennett, C. W.
1934. plant-tissue relations of the sugar-beet curly-top virus. Jour. Agr. Research 48: 665–701, illus.

(5) Clinch, P.
1932. cytological studies of potato plants affected with certain virus diseases. Roy. Dublin Soc. Sci. Proc. (n. s.) 20: 143–172, illus.

(6) Dufrenoy, J., and Dufrenoy, M. L.
1934. cytology of plant tissues affected by viroses Phytopathology 24: 599–619, illus.

(7) Edson, H. A.
1915. histological relations of sugar-beet seedlings and phoma betae. Jour. Agr. Research 5: 55–58, illus.

(8) Esau, K.
1934. cell degeneration in relation to sieve-tube differentiation in curly-top beets. a preliminary note. (Phytopath. Note) Phytopathology 24: 303–305, illus.

(9) ———
1934. ontogeny of phloem in the sugar beet (beta vulgaris l.). Amer. Jour. Bot. 21: 632–644, illus.

(10) Esau, K.
1935. ONTOGENY OF THE PHLOEM IN SUGAR BEETS AFFECTED BY THE CURLY-TOP DISEASE. Amer. Jour. Bot. 22: 149–163, illus.
(11) ———
1935. INITIAL LOCALIZATION AND SUBSEQUENT SPREAD OF CURLY-TOP SYMPTOMS IN THE SUGAR BEET. Hilgardia 9: 397–436, illus.
(12) Goldstein, B.
1928. NUCLEAR FORM AS RELATED TO FUNCTIONAL ACTIVITIES OF NORMAL AND PATHOLOGICAL CELLS. Bot. Gaz. 86: 365–383, illus.
(13) Iwanowski, D.
1903. ÜBER DIE MOSAIKKRANKHEIT DER TABAKSPFLANZE. Ztschr. Pflanzenkrank. 13: 1–41, illus.
(14) Lee, A. B.
1928. BOLLES LEE'S MICROTOMIST'S VADE-MECUM; A HANDBOOK OF THE METHODS OF MICROSCOPIC ANATOMY. Ed. 9, edited by J. B. Gatenby and E. V. Cowdry . . . 710 pp., illus. Philadelphia.
(15) Rawlins, T. E.
1926. CYTOLOGY OF ROOT TIPS FROM SUGAR BEETS HAVING THE CURLY-TOP DISEASE. (Abstract) Phytopathology 16: 761.
(16) Schander, R.
1930. BERICHT ÜBER DIE TÄTIGHEIT DES INSTITUTS FÜR PFLANZENKRANKHEITEN 1929/30. Landw. Jahrb. 72 (Ergänzungsbd. 1): 62–91, illus.
(17) Sharp, L. W.
1934. INTRODUCTION TO CYTOLOGY. Ed. 3, 567 pp., illus. New York and London.
(18) Tischler, G.
1934. HANDBUCH DER PFLANZENANATOMIE. 628 pp., illus. Berlin.

RELATIVE SUSCEPTIBILITY OF CERTAIN SPECIES OF GRAMINEAE TO CERCOSPORELLA HERPOTRICHOIDES [1]

By RODERICK SPRAGUE

Assistant pathologist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

Cercosporella herpotrichoides Fron, which causes an important foot rot of winter cereals in the Pacific Northwest and in northwestern continental Europe, has been known to mycologists since 1912 (*6*),[2] but its importance as a parasite of winter cereals was determined much more recently (*2*, *3*, *4*, *5*, *13*, *16*). The published information on the host range of *C. herpotrichoides* is largely confined to brief reports on the relative susceptibility of cultivated varieties of winter wheat (*Triticum aestivum* L.) and winter barley (*Hordeum vulgare* L.). Sprague and Fellows (*16*) and Sprague [3] have shown that certain types of wheat are more severely attacked in the field than others and that most varieties of barley are more resistant to the fungus than is wheat but less so than oats (*Avena sativa* L.) and certain other grains. Schaffnit (*12*) reported that wheat and barley are susceptible to *C. herpotrichoides*, and he found definite correlation between the prevalence of "straw breaking" and the development of the sclerenchymatous structure of the plant. Moritz and Brockmann (*10*), who were apparently the first to recognize the disease in Germany, confined their study of *C. herpotrichoides* to wheat. Foëx and Rosella (*4*) listed the fungus as parasitic on all of a large number of wheat varieties that were tested, on two-row, "four-row", and six-row barleys, on several species of *Avena*, and on Broekema rye. Recently Foëx (*1*) reported that *C. herpotrichoides* infected nine species of *Triticum* and six species of *Hordeum*, and *Avena orientalis* only slightly. Nielsen (*11*) reported that *C. herpotrichoides* had been found on wheat in Denmark.

Data are presented on the relative susceptibility of a number of wild and cultivated gramineous hosts to *Cercosporella herpotrichoides* and on the results of experimental studies in the greenhouse at Corvallis, Oreg., and at the Arlington Experiment Farm, Arlington, Va. near Washington, D. C. The latter indicate that many of the field-grown, so-called resistant cereals are actually not resistant but escape only by virtue of certain timely climatological factors. Evidence also is presented of the possible source of the disease from the original sod-grass prairie sections of the Columbia Basin.

[1] Received for publication May 5, 1936; issued Nov. 1, 1936. Cooperative investigations by the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Oregon, Idaho, and Washington Agricultural Experiment Stations.

[2] Reference is made by number (italic) to Literature Cited, p. 669.

[3] SPRAGUE, R. CERCOSPORELLA FOOT ROT OF WINTER GRAINS IN THE PACIFIC NORTHWEST. U. S. Dept. Agr. Bur. Plant. Indus. [Unnumbered Pub.] 10 pp. Aug. 17, 1934. [Mimeographed.]

NATURAL HOST RANGE

In the United States the cercosporella foot rot is almost entirely confined to sod-grass prairies whereon a certain specific native vegetation originally occurred (*14*). Sprague and Fellows (*16*) recently reported that in the Pacific Northwest *Cercosporella herpotrichoides* had not been isolated from any range or pasture grass, although lesions resembling those caused by it on cereals were found on *Agropyron inerme* (Scribn. and Smith) Rydb. The original collection on this grass was made in June 1929 at the margin of a field of winter wheat (Hybrid 128) in the Spokane River Valley of Washington. Since that date further collections of this type of injury on this grass were made in June 1931, 1932, and 1933 at the same general location. In 1933, typical cercosporella lesions were also found on *A. riparium* Scribn. and Smith, *Koeleria cristata* (L.) Pers., and *Poa secunda* Presl. (*P. sandbergii* Vasey) in the Spokane Valley. Pure cultures of *C. herpotrichoides* were obtained in 1933 from all of these species of grasses except *P. secunda*, but with considerable difficulty, as the faster growing *Wojnowicia graminis* (McAlp.) Sacc. and D. Sacc. was predominant in the lesions on the hosts at this season (*15*). During the same month typical eyespot lesions of cercosporella were found on the culms of *Bromus tectorum* L., which occurred as a weed in cercosporella-infested wheat fields on Peone Prairie, Spokane County, Wash. Pure-culture isolations of *C. herpotrichoides* were easily obtained from this host. This same grass was found affected at Friend, Wasco County, Oreg., and in Union County, Oreg., in 1934.

In June 1934, following an exceptionally mild, almost frost-free winter, typical cercosporella lesions were found on *Bromus inermis* Leyss., *B. marginatus* Nees, and *Sitanion hystrix* (Nutt.) J. G. Smith near Nezperce, Idaho. These grasses were growing at the edge of fields of winter wheat heavily infested with *Cercosporella herpotrichoides*.

As reported in a check list of diseases on Oregon Gramineae,[4] *Cercosporella herpotrichoides* attacked, in the field, *Aegilops cylindrica* Host, *A. ovata* L., *A. triuncialis* L., *Bromus tectorum* L., winter oats (*Avena sativa*), rye, wheat, einkorn, spelt, and emmer.

Those mentioned above represent the only collections of *Cercosporella herpotrichoides* made on naturally occurring range and pasture grasses in the United States during 7 years of intensive search.

EXPERIMENTS IN NATURALLY INFESTED FIELDS

CEREALS

A brief summary of the field studies conducted in the Pacific Northwest with respect to the susceptibility of wheat and barley varieties to *Cercosporella herpotrichoides* has been published (*16*). This report stated that "the relative susceptibility of varieties varies somewhat from year to year." This point was clearly brought out during the long growing season of 1933–34 in the Columbia Basin, when certain varieties that were previously somewhat "resistant" became virtually as susceptible as the average susceptible varieties.

[4] SPRAGUE, R. A PRELIMINARY CHECK LIST OF THE PARASITIC FUNGI ON CEREALS AND OTHER GRASSES IN OREGON. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Reptr. 19: 156–186. 1935. [Mimeographed.]

In order to facilitate comparison of the susceptibility of cereals grown in the field with those grown in the greenhouse, the relative susceptibility of a number of representative varieties is given in table 1 for the period from 1930 to 1935. The results will be considered later under the heading "Discussion."

TABLE 1.—*Disease reaction of certain varieties of cereals to Cercosporella herpotrichoides for the period 1930–35*

[Hybrid 128 rated as 100]

Host (species and variety)	C. I.[1] no.	Reaction to *Cercosporella herpotrichoides*						
		1930	1931	1932	1933	1934	1935	Average
Arena sativa L. (oats):								
Winter Turf (Gray Winter)	1570	2	4	2	4	15	4	5
Hordeum vulgare L. (barley):								
Alaska	4106	95	100	95	85	105	90	95
Tennessee Winter	257	90	83	.....	.....	.....	.....	87
Winter Club	592	50	48	50	50	74	65	57
Olympia	6107	.....	.....	.....	.....	72	70	71
Secale cereale L. (rye):								
Oregon Winter	.....	5	5	8	5	10	.....	6
Triticum dicoccum Schrank (emmer):								
Black Winter	2337	30	29	.....	.....	.....	.....	29
Triticum durum Desf. (durum):								
Akrona	6881	101	.....	.....	.....	.....	.....	.....
Kubanka	1440	103	101	.....	.....	.....	.....	.....
Triticum monococcum L. (einkorn)	2433	9	.....	.....	.....	.....	.....	.....
Triticum spelta L. (spelt):								
Alstroum	1773	.....	140	130	.....	.....	.....	135
Triticum aestivum L. (common wheat):								
Federation	4734	80	78	90	88	95	90	87
Goldcoin	4156	105	115	113	110	115	115	112
Hohenheimer	11458	65	63	68	.....	.....	.....	65
Hosar	10067	75	74	80	.....	.....	.....	76
Kanred×Marquis	8245	.....	97	98	140	80	105	104
Kharkof	8249	110	120	110	115	120	120	116
Ridit	6703	100	93	103	100	95	.....	98
Turkey	4429	110	120	107	120	120	115	115
Wilhelmina (Holland)	11389	85	68	65	.....	90–110	90	81
Triticum compactum Host (club wheat):								
Hybrid 63	4510	110	111	107	100	105	100	105
Hybrid 128	4512	100	100	100	100	100	100	100
Hybrid 143	4160	95	97	105	95	110	98	100
Triticum aestivum×*Secale cereale* (wheat×rye):								
Meister's amphidiploid	11403	.....	.....	.....	T	24	.....	.....

[1] C. I. refers to accession number of the Division of Cereal Crops and Diseases, formerly Office of Cereal Investigations.

OTHER GRASSES

One point of increasing importance in connection with any program for control of the cercosporella foot rot is the need for information on the relative susceptibility of various range-land or pasture grasses to *Cercosporella herpotrichoides*. Recent developments in connection with the adjustment of the acreage sown to wheat, together with a growing conviction that other crops must be rotated with wheat on cercosporella-infested soil, have increased the interest in the growing of grasses in these prairie sections. This has led to inquiries as to the susceptibility of various grasses to *C. herpotrichoides*, and although all field observations indicated that the sparsely seeded grasses would not become injured to any extent by the fungus, certain field trials were conducted to obtain definite information on this point.

A number of grasses were seeded in the regular cereal test plot in 16-foot rows spaced 1 foot apart on September 25, 1933, in cercosporella-infested soil at High Prairie, Wash. On February 5, 1934,

healthy mature plants of crested wheatgrass, *Agropyron cristatum* (L.) Beauv., were obtained from D. E. Stephens, Sherman County Branch Station, Moro, Oreg., and transplanted into cercosporella-infested soil at High Prairie. Some of these were planted in the infested wheat and others a short distance away in an unseeded plot. Winter wheat was also transplanted from Moro to the unseeded plot at the same time. The results of this experiment (table 2) indicate that these grasses, which are necessarily sown in widely spaced rows or thinly broadcast in the semiarid cercosporella region, will not become heavily infected with cercosporella foot rot. It is shown in table 2

TABLE 2.—*Disease reaction of certain Gramineae to Cercosporella herpotrichoides at High Prairie, Wash.*

[Records taken June 6, 1934]

Host (species and variety)	Method of propagation	Culms		Estimated loss
		Height	Number	
		Inches		*Percent*
Arrhenatherum elatius (L.) Mert. and Koch.	Seeded in cereal plot	16	120	0
Agropyron cristatum	Transplanted to cereal plot	16	42	5.3
Do	Transplanted to unseeded plot	16	60	0
Bromus secalinus L.	Seeded in cereal plot	10	151	0
Festuca elatior L.	do	3	75	0
Festuca rubra L.	do	3	137	0
Hordeum vulgare (Winter Club)	do	34	81	49.2
Triticum compactum (Hybrid 128)	do	33	181	68.3
Do	Transplanted to cereal plot	29	165	22.3
Do	Transplanted to unseeded plot	28	179	2.5

that crested wheatgrass, while susceptible when grown under favorable conditions of humidity among the wheat plants, was healthy when grown in the open. This work was done during the most favorable season on record for the foot rot. It has been observed many times and repeatedly proved that even the most susceptible varieties of wheat may be kept almost free of foot rot by widely spaced seeding.

EXPERIMENTS IN THE GREENHOUSE

METHOD OF PROCEDURE

Most of the studies of *Cercosporella herpotrichoides* were conducted at Corvallis, Oreg., during the cooler months (November to April) in an unheated greenhouse maintained at a temperature ranging mostly between 5° and 18° C. The relative humidity was maintained above 85 percent for the most part, as any prolonged period with humidity below 70 percent distinctly retards the parasitic activity of *C. herpotrichoides*.

Some studies were made at the Arlington Experiment Farm, Arlington, Va., in a greenhouse in which the temperature was similar to that in the greenhouse at Corvallis. Usually the relative humidity was kept above 95 percent by the use of a mist spray. The plants at Arlington Farm were grown under 250-watt lights from 4 p. m. until midnight during each 24-hour period.

Plants were grown in somewhat infertile, unsterilized, cercosporella-free Newberg sandy loam at Corvallis or in a mixture of two-thirds unsterilized river sand and one-third loam at Arlington Farm. The inoculum consisted of active cultures of *Cercosporella herpotrichoides* grown on boiled and quadruple-sterilized wheat kernels in liter or half-liter Erlenmeyer flasks. In the majority of the artificial inoculation experiments from 1929 to 1933 the inoculum was added to the soil at seeding time. The plants were grown either in cubic-foot boxes or in benches having a soil depth of 8 inches. The inoculum was placed above the seed with one-half inch of soil intervening and was applied at the rate of four to six of the fungus-covered grains of inoculum to each cereal seed or large grass seed sown. This method of inoculation induced an extremely severe foot rot with abnormally pronounced stunting of infected culms. By using this method, however, it was possible to obtain information on the true susceptibility of certain varieties of cereals and on various range and pasture grasses that usually escape severe injury in the field because of such conditions as late maturity or reduced humidity brought about by sparse stand.

In most of the more recent inoculation studies with *Cercosporella herpotrichoides* the inoculum was laid at the base of the culms about 60 days after the emergence of the plants and was then lightly covered with moist soil. This method was particularly necessary in handling grasses with small seeds, because otherwise the mass of inoculum was somewhat toxic to the germinating seeds. The addition of inoculum to the plant after it has become established more nearly approaches the manner of infection in the field in the Pacific Northwest and results in less stunting of the plant than when the inoculum is applied at seeding time.

Several attempts to use conidia of *Cercosporella herpotrichoides*, grown on corn meal, for inoculation purposes were not consistently successful on winter wheat in the greenhouse, and the practice was discontinued.

The probable reduction in yield under greenhouse conditions due to *Cercosporella herpotrichoides* was calculated by means of a graduated scale in which the approximate reduction in potential yield was considered as follows: Plants dead by disease, 100 percent loss; very severely injured, 80 percent loss; severely injured, 60 percent loss; moderately injured, 40 percent loss; slightly injured, 20 percent loss. This scale differs somewhat from that used in the field (*16*) because of the severe injury and stunting encountered under optimum greenhouse conditions. It must be emphasized that the injury under the greenhouse conditions was very severe and losses up to 80 percent of the potential yield were not uncommon; in a few cases the plants were nearly all killed.

RESULTS

The results of inoculation studies with *Cercosporella herpotrichoides* on a number of gramineous hosts during the period 1930–31 in the greenhouse at Corvallis, Oreg., are presented in table 3. The plants were sown and inoculated on two dates, October 7, 1930, and January 10, 1931, and were harvested on January 18, 1931, and May 4, 1931.

TABLE 3.—*Disease reaction of cereals and other grasses inoculated with pure cultures of Cercosporella herpotrichoides in the greenhouse at Corvallis, Oreg., 1930–31*

Host (species and variety)	C. I. no.	Date sown	Culms showing indicated degree of injury							Estimated loss
			Healthy	Slight	Moderate	Severe	Very severe	Dead	Total	
			No.	No.	No.	No.	No.	No.	No.	Pct.
Aegilops triuncialis	------	Oct. 7	14	58	29	------	------	------	101	22.9
Avena byzantina C. Koch (oats):										
Kanota	839	Jan. 10	23	------	------	------	------	------	23	.0
Red Rustproof	------	do	21	2	2	1	------	------	26	5.0
Avena fatua L. (wild oats)	------	do	34	------	------	------	------	------	34	.0
Avena sativa L. (oats):										
Winter Turf	1570	Oct. 7	123	2	1	1	------	------	127	1.1
Hordeum vulgare (barley):										
Alaska	4106	do	41	24	28	7	4	1	105	23.2
Do	4106	Jan. 10	22	17	3	------	1	3	46	18.3
Tennessee Winter	257	do	23	12	6	3	2	4	50	24.4
Winter Club	592	Oct. 7	25	78	28	13	6	------	150	25.4
Secale cereale (rye):										
Oregon Winter	------	do	45	------	------	------	------	------	45	.0
Do	------	Jan. 10	21	------	------	------	------	------	21	.0
Triticum dicoccum (emmer):										
Black Winter	2337	Oct. 7	11	12	40	10	3	1	77	36.1
Do	2337	Jan. 10	11	11	3	------	------	------	25	13.6
Triticum durum (durum):										
Akrona	6881	Oct. 7	22	7	10	3	2	1	45	21.8
Kubanka	1440	Jan. 10	9	2	6	4	------	3	24	34.1
Triticum monococcum (einkorn)	2433	Oct. 7	19	37	55	------	------	------	111	26.5
Triticum spelta (spelt):										
Alstroum	1773	do	11	9	10	28	36	10	104	62.9
Do	1773	Jan. 10	6	5	8	18	2	1	40	44.0
Triticum aestivum (common wheat):										
Buffum No. 17	3330	Oct. 7	10	16	29	42	36	41	174	63.1
Federation	4734	do	3	9	11	8	7	12	50	61.2
Goldcoin (Fortyfold)	4156	do	2	3	24	25	13	4	71	55.8
Harvest Queen	5314	do	17	6	51	49	24	7	154	50.1
Hohenheimer	11458	do	2	17	37	29	27	6	118	53.6
Do	11459	do	2	0	22	30	15	10	79	61.7
Hosar	10067	do	2	5	23	35	27	8	100	60.8
Minhardi×Minturki	8215	do	2	1	18	15	39	7	82	66.5
Purplestraw	1915	do	1	5	24	28	21	40	119	77.2
Turkey	4429	do	40	14	12	20	32	14	132	44.9
Wilhelmina (Holland)	11389	do	0	5	32	28	17	11	93	59.4
Triticum compactum (club wheat):										
Hybrid 128	4512	do	15	6	21	21	21	9	93	51.6
Hybrid 143	4160	do	27	10	16	6	5	6	70	38.3
Jenkin	5177	do	7	2	9	8	6	0	32	42.5
Triticum aestivum×*Secale cereale*:										
Wheat-rye	8890	do	5	7	5	24	47	2	90	63.8

A similar experiment, which is summarized in table 4, was conducted in 1934–35. The grasses were seeded October 7 in the benches and inoculated after emergence on November 15 with pure-culture inoculum and in addition with cercosporella-infested soil taken from the surface soil of a heavily infested field at High Prairie, Wash. The plants were grown under 250-watt lights from 5 p. m. until 8 a. m. of each 24-hour period. The final records were taken February 19, 1935.

Other experiments on the resistance of species of Gramineae to *Cercosporella herpotrichoides*, which dealt especially with range and pasture grasses, were made at Corvallis and at the Arlington Farm in the greenhouse. The results of these experiments are given in tables 5, 6, and 7. The results presented in table 5 are from several species of Gramineae seeded October 1, 1931, in Newburg sandy loam soil in

rows spaced 4 inches apart in the greenhouse at Corvallis, Oreg. The fungus was reisolated from diseased plants of each host. All uninoculated checks remained healthy.

TABLE 4.—*Disease reaction of cereals and other grasses inoculated with pure cultures of Cercosporella herpotrichoides, and grown on soil infested with that organism, in the greenhouse at Corvallis, Oreg., 1934–35*

Host (species and variety)	C. I. no.	Culms showing indicated degree of injury							Estimated loss
		Healthy	Slight	Moderate	Severe	Very severe	Dead	Total	
		Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Percent*
Aegilops triuncialis	------	4	4	10	52	5	8	83	42.4
Do	------	------	------	------	52	11	30	93	64.1
Avena byzantina (oats):									
Kanota	839	2	7	26	38	2	------	75	30.4
Do	839	2	2	6	38	12	2	62	39.0
Red Rustproof	------	12	28	16	7	2	------	65	16.0
Do	------	------	1	5	39	18	1	64	43.8
Avena fatua (wild oats)	------	10	18	17	15	------	------	60	18.6
Do	------	1	1	14	52	6	------	74	38.5
Avena sativa (oats):									
Winter Turf	1570	14	16	19	45	5	------	99	25.6
Do	1570	5	2	5	53	12	------	77	41.5
Hordeum vulgare (barley):									
Alaska	4106	3	6	12	33	6	13	73	60.3
Do	4106	------	------	------	48	8	15	71	57.4
Tennessee Winter	257	------	1	2	12	2	------	17	40.6
Do	257	------	------	1	10	------	9	20	66.0
Winter Club	592	------	5	15	52	------	1	73	34.7
Do	592	------	------	------	56	5	3	64	46.0
Winter (Wash. no. 2801)	------	10	12	36	23	2	3	86	25.8
Do	------	1	3	26	39	2	4	75	30.2
Secale cereale (rye):									
Oregon Winter	------	5	------	5	5	------	------	15	20.0
Do	------	4	1	4	4	------	------	13	19.2
Triticum dicoccum (emmer):									
Black Winter	2337	1	1	3	47	4	3	59	43.5
Do	2337	------	1	1	65	------	------	67	39.2
Triticum durum (durum):									
Akrona	6881	3	2	13	67	14	1	100	41.8
Kubanka	1440	6	2	9	31	5	2	55	37.1
Triticum monococcum (einkorn)	2433	5	6	32	41	------	4	88	31.1
Triticum spelta (spelt):									
Alstroum	1773	7	3	13	79	5	1	108	36.5
Do	1773	3	2	2	55	14	5	81	48.0
Bearded winter	------	1	1	2	47	25	13	89	58.7
Triticum aestivum (common wheat):									
Federation	4734	------	------	3	48	12	3	66	48.6
Goldcoin (Fortyfold)	4156	------	------	------	10	30	51	91	86.8
Hohenheimer	11458	1	3	5	43	26	42	120	67.7
Hosar	10067	7	6	4	55	13	8	93	44.9
Minhardi × Minturki	8215	2	5	9	56	25	84	181	71.1
Purplestraw	1915	------	------	------	30	10	40	80	75.0
Ridit	6703	------	------	------	26	33	29	88	74.7
Turkey	4429	------	1	3	25	31	64	124	88.3
Wilhelmina (Holland)	11389	------	1	------	29	16	28	74	70.9
Triticum compactum (club):									
Hybrid 128	4512	3	5	5	53	13	4	83	44.7
Do	4512	------	------	4	61	8	6	79	47.6
Do	4512	------	------	1	53	8	6	68	49.7
Do	4512	------	2	3	45	10	7	67	50.4
Do	4512	------	------	------	54	5	14	73	54.2
Do	4512	------	------	------	42	8	10	60	54.5
Do	4512	------	------	------	68	4	25	98	56.5
Do	4512	1	------	------	38	5	14	59	56.7
Do	4512	------	1	1	46	25	10	81	59.7
Do	4512	------	------	------	28	33	10	75	64.0
Do	4512	1	1	2	28	39	14	82	68.9
Do	4512	------	1	------	41	3	1	67	32.6
Hybrid 143	4160	9	5	8	71	10	------	85	43.6
Jenkin	5177	------	1	3					

TABLE 5.—*Disease reaction of several species of Gramineae inoculated with pure cultures of Cercosporella herpotrichoides in the greenhouse at Corvallis, Oreg., 1931–32*

Host	Culms showing indicated degree of injury							Estimated loss
	Healthy	Slight	Moderate	Severe	Very severe	Dead	Total	
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Percent*
Agropyron repens (L.) Beauv	44	15		1			60	6.0
Agrostis palustris Huds	100		1	1	2		104	2.5
Bromus mollis L	40	4	2	1			47	5.5
B. rigidus Roth	55	22					77	5.7
B. secalinus	14	7	6	27	26	14	94	58.3
B. tectorum	25	19	3				47	10.6
Lolium multiflorum Lam. (imported)	65	25	15	16	2		123	18.7
L. multiflorum (western)	17	21	21	27	4		90	35.5
Triticum monococcum (einkorn)	4	57	7				68	20.9
T. aestivum (Kharkof)	1	3	4	5	5	15	33	73.3

A number of additional species of grasses were seeded in Newburg sandy loam soil in greenhouse benches at Corvallis, Oreg., on October 19, 1933. The rows were spaced 7 inches apart. Inoculum was added to the soil around the plants on December 18, and they were harvested March 14, 1934. Results from this test are given in table 6. Reisolations were positive from all hosts that showed more than 1 percent average loss. All uninoculated check plants remained healthy.

TABLE 6.—*Disease reaction of several species of Gramineae inoculated with pure cultures of Cercosporella herpotrichoides in soil in the greenhouse at Corvallis, Oreg., 1933–34*

Host	Reaction to inoculum from—			
	Triticum aestivum		*Bromus tectorum*	
	Infected culms	Estimated loss	Infected culms	Estimated loss
	Number	*Percent*	*Number*	*Percent*
Agropyron cristatum	55	12.1	124	10.0
A. intermedium (Host) Beauv	54	8.8		
A. smithii Rydb	106	12.5		
A. spicatum (Pursh) Scribn. and Smith	63	5.8	11	25.4
A. subsecundum (Link) Hitchc	34	7.9		
A. tenerum Vasey	63	6.2		
A. violaceum (Hornem.) Lange	78	18.7		
Agrostis alba L	199	0	212	0
Bromus rigidus	168	2.2		
B. secalinus	100	3.6		
B. tectorum	116	4.3	97	5.2
Cynosurus cristatus L	187	.5	156	0
Dactylis glomerata L	111	0	85	0
Festuca elatior			97	4.3
F. ovina L	94	0	92	3.0
F. rubra fallax Hack	143	0	172	
Lolium multiflorum			98	3.5
L. perenne L			103	4.4
Phalaris arundinacea L	82	.1	67	0
Phleum pratense L	177	0	105	0
Poa nemoralis L	145	.1	158	0
Poa secunda Presl			383	3.1
Poa trivialis L	235	0		
Triticum monococcum	87	17.1		
T. aestivum (Golden)	145	47.1		
T. aestivum (Huston)			56	52.5
T. aestivum (Hybrid 128)	96	51.6	129	56.6

A number of species of *Aegilops*, *Haynaldia*, and *Triticum* were planted in soil in the greenhouse at Arlington Farm on November 12, 1932, inoculated in the usual way with pure cultures of *Cercosporella herpotrichoides*, and harvested February 17, 1933.[5] The infection data are given in table 7.

TABLE 7.—*Disease reaction of several species of Gramineae inoculated with pure cultures of Cercosporella herpotrichoides in the greenhouse at the Arlington Experiment Farm, Arlington, Va., 1932–33*

Host	Culms showing indicated degree of injury							Estimated loss
	Healthy	Slight	Moderate	Severe	Very severe	Dead	Total	
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Percent*
Aegilops aucheri Boiss. var. *virgata*		2	4	20	8	3	37	63.2
A. bicornis (Forsk.) Jaub. and Sp.	1	5	6	3	5	5	25	56.8
A. ovata L. var. *umbonata* Zhuk.	4	12	7	3			26	27.0
A. squarrosa L. var. *typica*	22	11	3				36	9.4
A. tauschii Coss.		3	6	4	1	7	21	62.9
A. triaristata Willd.	6	10	3				19	16.8
A. triuncialis	12	20	11	8	2		53	27.9
A. uniaristata Vis.	15	5	6	2		1	29	16.0
A. variabilis Eig.					1	7	8	97.5
A. ventricosa Tausch	55	2	1	1			59	2.5
Haynaldia villosa (L.) Shur.	51	6	1				58	2.7
Triticum dicoccum [1]				7	8	21	36	87.7
T. dicoccum var. Vernal				3	11		14	75.2
T. dicoccoides Koern.				13	2	1	16	65.0
T. orientale Percival				4	2		6	66.7
T. persicum Vav. var. *rubiginosum*				4	1	4	9	80.0
T. timopheevi Zhuk. var. *veliculosum*				6	2	7	15	81.3
T. aestivum var. Hybrid 128	1	9	13	16	4	5	48	51.6
T. aestivum var. Chinese			5	12	4	9	30	71.3
T. aestivum × *Secale cereale* [2]	18	2					20	2.2

[1] Sando's awnless emmer.
[2] Meister's amphidiploid.

POSSIBLE ORIGIN OF CERCOSPORELLA FOOT ROT IN THE PACIFIC NORTHWEST

The first authentic collection of cercosporella foot rot in the United States was made on one of the prairies northeast of Hillyard near Spokane, Wash., in 1919 (*7*). The disease was reported a short time later from Klickitat County, Wash. (*8*), and Union County, Oreg. (*9*), and since then from a number of localities (*14*, *16*).[6] Pioneer wheat growers are agreed that the disease was not widespread before 1910 and when present occurred on north slopes near the bottom of draws where moisture was relatively abundant but not excessive. All evidence seems to indicate that the disease has been present for a long period on native grasses in the Columbia Basin and that the causal fungus has adapted itself to the more humid conditions of the wheat fields from a former struggling existence on native grasses where it had persisted even under semiarid conditions. The fact that the disease is closely confined to certain isolated even though moderately extensive areas of a certain vegetation type in the Pacific Northwest strongly supports this view.

In the greenhouse all the species of *Agropyron* that were inoculated were susceptible to *Cercosporella herpotrichoides*, this grass ranking

[5] The seed for these experiments was furnished by W. J. Sando, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.
[6] See footnote 3.

as one of the most susceptible of all the grasses tested. It is possible, therefore, that *A. spicatum* and *A. inerme* may be native hosts of this fungus.

DISCUSSION

RELATIVE SUSCEPTIBILITY OF CEREALS

The field and greenhouse studies show not only that certain varieties of wheat are highly susceptible to *Cercosporella herpotrichoides* but that those varieties that do escape disease in the field are often readily attacked by the fungus under conditions more favorable for the disease. For example, Wilhelmina, which is a late-maturing wheat with heavy straw, normally escapes serious injury in the field, but under optimum conditions for the fungus such as prevailed in the greenhouse at Corvallis it becomes seriously diseased. Following the mild, open winter of 1933–34 Wilhelmina and Red Russian, which seldom show serious injury in the field, were heavily diseased in the fields near Nezperce, Idaho. This tends to confirm the results of the greenhouse studies. In the greenhouse the same conditions held true for other late-maturing varieties of wheat, such as Hohenheimer and Hosar.

When sufficient inoculum is added to the soil such cereals as oats or einkorn and Oregon winter rye, which usually show little injury in the field, become heavily infected (table 4).

The value of the greenhouse studies lies in their disclosing the lack of true resistance in certain varieties in the field, information that is important in a breeding program.

RELATIVE SUSCEPTIBILITY OF OTHER GRASSES

As shown in tables 3 to 6, many species of grasses are susceptible to *Cercosporella herpotrichoides* when grown in crowded rows under optimum conditions for infection in the greenhouse. In general, however, the range and pasture grasses are distinctly more resistant than most varieties of wheat, and some wiry-leaved grasses, such as a number of species of *Festuca*, are highly resistant. Certain of the wheat relatives listed in table 6 also seem to show some resistance. While the number of culms that were tested in this experiment was very small, the conditions were so nearly ideal for maximum development of the disease that the results are considered as strongly suggestive. Some of these wheat relatives, such as *Aegilops ventricosa*, may have some value in breeding for resistance to cercosporella foot rot. This process would be very slow and uncertain, however, inasmuch as field experience with cereals bearing *Aegilops* parentage has been somewhat discouraging. Most of these hybrids that show resistance to cercosporella foot rot produce low yields, and those that produce fair yields assume the susceptible characteristics of the wheat parent.

On account of the need for widely spaced seeding in the Columbia Basin, in addition to the moderate resistance of the species, most of the adapted grasses other than cereals can be sown on cercosporella-infested ground with little danger of important losses from cercosporella foot rot.

SUMMARY

Cercosporella herpotrichoides Fron has been isolated from a few wild grass hosts growing in or at the edge of fields of winter wheat that were severely infested with the fungus.

The reaction to *Cercosporella herpotrichoides* of a number of cereals and other grasses in the field is presented in tabular form for each year for the period 1930–35. Because of semiarid conditions it is necessary to seed grasses sparsely. This assists aeration and precludes any great danger of loss from *C. herpotrichoides*, notwithstanding the moderate susceptibility of these grasses under greenhouse conditions optimum for the disease.

The results obtained in the greenhouse under optimum conditions for the disease were of special value in showing that certain late-maturing varieties of cereals that appeared resistant under field conditions in reality escaped the disease and do not contain genetic factors for resistance. The so-called resistance of these varieties under field conditions is considered to be due to escape through their lateness in maturing.

Various species of *Aegilops* and *Triticum* showed great differences in reaction to *Cercosporella herpotrichoides*, some being markedly resistant and some very susceptible. There is a possibility that resistant cereals may eventually be developed from hybrids between wheat and certain related wild grasses.

In the greenhouse under conditions optimum for the disease a number of genera and species of grasses were moderately susceptible to *Cercosporella herpotrichoides*. Species of *Agropyron* were found to be particularly susceptible.

Indications are that cercosporella foot rot has been present for a long period on grasses native to the prairies of the Columbia Basin.

LITERATURE CITED

(1) Foëx, E.
1935. QUELQUES OBSERVATIONS SUR LES MALADIES DU PIED DES CÉRÉALES. Compt. Rend. Acad. Agr. France 21: 501–505.

(2) ——— and Rosella, E.
1930. SUR LES DIVERSES FORMES DU PIÉTIN. Rev. Path. Vég. et Ent. Agr. 17: [41]–51.

(3) ——— and Rosella, E.
1930. RECHERCHES SUR LE PIÉTIN. Ann. Epiphyties 16: [51]–82, illus.

(4) ——— and Rosella, E.
1932. RECHERCHES SUR LE PIÉTIN DU BLÉ AU COURS DE LA CAMPAGNE 1930–1931. Compt. Rend. Acad. Agr. France 18: 376–383.

(5) ——— and Rosella, E.
1934. ÉTUDE SUR LES PIÉTINS DES CÉRÉALES. Compt. Rend. Acad. Agr. France 20: 480–483.

(6) Fron, G.
1912. CONTRIBUTION À L'ÉTUDE DE LA MALADIE DU "PIED NOIR DES CÉRÉALES" OU "MALADIE DU PIÉTIN." Ann. Sci. Agron. Franç. et Étrang. (4) 1: 3–29, illus.

(7) Heald, F. D.
1920. INVESTIGATIONS NEEDED. Wash. Agr. Expt. Sta. Bull. 155 (Ann. Rept. 29): 38.

(8) ———
1922. THE PLANT DISEASE SURVEY. Wash. Agr. Expt. Sta. Bull. 167 (Ann. Rept. 31): 41–42.

(9) McKinney, H. H.
1925. FOOT-ROT DISEASES OF WHEAT IN AMERICA. U. S. Dept. Agr. Bull. 1347, 40 pp., illus.

(10) Moritz, O., and Bockmann, H.
1933. Einleitende studien über cercosporella herpotrichoides fron. Angew. Bot. 15: [409]–419, illus.
(11) Nielsen, O.
1934. Oversigt over plantesygdomme. 197.— Vintermaanedern og April 1934. Statens Plantepatol. Forsøg. 5 pp.
(12) Schaffnit, E.
1933. Beiträge zur kenntnis der fusskrankheiten des getreides. (I. mitteilung) cercosporella herpotrichoides (fron) als ursache der halmbruchkrankheit des getreides. Phytopath. Ztschr. 5: [493]–503, illus.
(13) Sprague, R.
1931. Cercosporella herpotrichoides fron, the cause of the columbia basin footrot of winter wheat. Science (n. s.) 74: 51–53.
(14) ———
1934. The association of cercosporella herpotrichoides with the festuca consociation. Phytopathology 24: 669–676, illus.
(15) ———
1935. Wojnowicia graminis as a very weak, secondary parasite of winter cereals. Phytopathology 25: 405–415, illus.
(16) ——— and Fellows, H.
1934. Cercosporella foot rot of winter cereals. U. S. Dept. Agr. Tech. Bull. 428, 24 pp., illus.

INTRASPECIFIC AND INTERSPECIFIC AVERSION IN DIPLODIA[1]

By PAUL E. HOPPE[2]

Associate pathologist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

In the course of an extensive plating program involving the identification of fungi associated with the ear-rot diseases of dent corn, strains[3] of *Diplodia zeae* (Schw.) Lév.[4] were observed which in culture manifest intraspecific aversion, a phenomenon infrequently reported in fungi. It was observed that when adjacent colonies of *D. zeae* appeared in the culture plates one of two contrasting reactions occurred where the mycelia came together: The hyphae either intermingled freely, with a piling up or "drifting" of the mycelium at the line of juncture, or, more frequently, an antagonistic reaction took place in which growth at the tips ceased and was apparently followed by a dying back of the mycelium. This was followed by a darkening of the agar between the averting colonies, delimiting them cleanly from each other (fig. 1). The aversion phenomenon occurred upon all culture media tried, including potato-dextrose agar, oatmeal agar, and Coons', Richards', and Czapeks' media. Aside from the fundamental aspects of the problem, further studies on aversion in this economically important fungus seemed justified on the basis of practical considerations, especially in relation to pathogenicity studies and inoculation technique. The present paper includes the results obtained in studies involving (1) stability of strains of *D. zeae* for aversion reactions, (2) number and distribution of strains, and (3) inoculation experiments with single strains and mixtures of strains of *D. zeae* and *D. macrospora*.

OCCURRENCE OF INTRASPECIFIC AVERSION IN FUNGI

A study of the literature reveals a growing list of cases in which aversion has been observed within specific lines of fungi. The cases cited below are those which the writer believes bear analogy to the situation in *Diplodia* to the extent that the manifestation of the phenomenon appears to be dependent upon genetic differences in the cultures involved. Cayley (*3*)[5], in 1923, first directed particular attention to this phenomenon in her studies with *Diaporthe perniciosa*. In a later paper Cayley (*4*) cites, as additional cases, those described by Brunswik in *Coprinus fimetarius* and in *C. friesii;* by Vandendries in *C. micaceus, C. radians,* and *Panaeolus complanatus;* by Nakata in *Sclerotium rolfsii;* and by Mounce in *Fomes pinicola*. More recently

[1] Received for publication May 18, 1936; issued Nov. 1, 1936. Cooperative investigation by the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Wisconsin Agricultural Experiment Station.

[2] The author wishes to express his thanks to Eugene H. Herrling, of the Department of Plant Pathology, University of Wisconsin, for the photographs.

[3] The term "strain", as used in the present paper, refers to any *Diplodia* culture or group of cultures that shows aversion to all other *Diplodia* cultures.

[4] The aversion phenomenon also has been observed in *Diplodia macrospora* Earle.

[5] Reference is made by number (italic) to Literature Cited, p. 679.

cases of intraspecific aversion have been reported by Vandendries in *Pleurotus columbinus* (*8*) and *P. ostreatus* (*9*), by Vandendries and Brodie (*10*) in *Lenzites betulina*, and by Brodie (*1*) in *Corticium calceum*.

STABILITY OF AVERSION REACTIONS OF STRAINS OF DIPLODIA ZEAE

Twenty-one cultures of *Diplodia zeae*, including 12 strains, all isolated from rotted kernels of dent corn, were used in tests for studying the stability of the particular aversion reactions. Stability of reaction was maintained in every culture, (1) following mycelial propagation, (2) through successive pycnidial generations, and (3) following reisolations from inoculated corn plants. Two-percent potato-dextrose agar was used in all plating tests on stability.

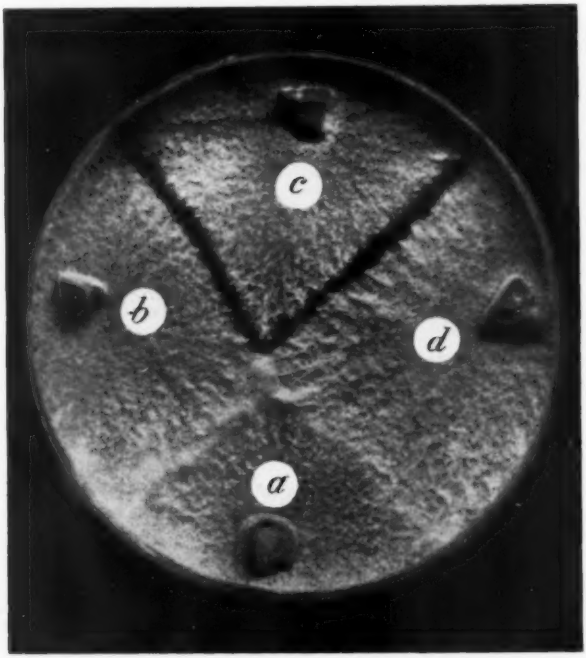


FIGURE 1.—Plate cultures showing aversion between strains of *Diplodia zeae*. Aversion is shown between colony *c* and colonies *b* and *d*. Colonies *a*, *b*, and *d* are of the same strain and show no aversion to one another. Colonies *a* and *c* would show aversion to each other if their mycelia came together. Note the intermingling or "drifting" of the mycelium between colonies *a* and *b* and between *a* and *d*.

The question first studied was whether two cultures of *Diplodia zeae* that show aversion to each other are of the same strain or of different strains. Plating mycelial inocula from any single culture of *D. zeae* at opposite sides in a Petri dish always resulted in a free intermingling of the hyphae, with a piling up or drifting of the mycelia where they met. In contrast, when mycelial inocula from two averting cultures were plated at opposite sides in a Petri dish, the characteristic

aversion reaction always occurred. It naturally followed that all isolates of a given strain, wherever obtained reacted alike toward other cultures. For example, if cultures A and B were found to be alike, and if C and D also were alike, then, if further platings showed A and C to be alike, it was invariably found that B and D likewise

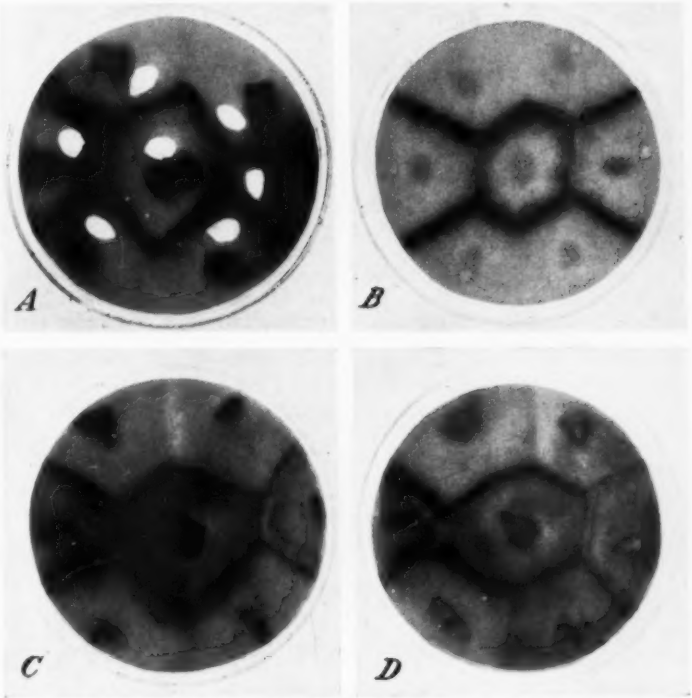


FIGURE 2.—Stability of aversion reactions in strains of *Diplodia zeae* as shown by plate cultures. *A*, Seven original colonies of *Diplodia* growing from corn kernels. *B*, The same cultures following propagation by means of mycelial transfers. *C*, Cultures again combined following three successive pycnidial generations. Monospore cultures were used throughout. *D*, The same cultures again combined following reisolations from inoculated ears of corn. The cultures in *C* were used for pycnospore production for the inoculations, therefore four pycnidial generations actually were involved in these stability studies.

would intermingle, and that any one of the four would react in exactly the same manner as any of the others when plated against any different strain.

STABILITY FOLLOWING MYCELIAL PROPAGATIONS

The 21 cultures used for the studies on stability following mycelial propagation were propagated in groups of 7 in each culture plate. The three groups involved five, four, and three different strains, respectively, which were so arranged on the plates that each group presented its characteristic design as a result of the reactions between the strains. Mycelial transfers to other plates, with the cultures placed in the same relative positions, resulted in every case in the same type of design as that in the original plate (fig. 2, *B*). This evidence

of stability was maintained in all cultures through each of three successive transfers.

STABILITY THROUGH SUCCESSIVE PYCNIDIAL GENERATIONS

After the cultures had been propagated three times by means of mycelial transfers, transfers were made to oatmeal agar to induce abundant pycnidial development. Three monosporic cultures were obtained from each of 3 randomly selected pycnidia from each of the 21 cultures. Plating the three cultures from a given pycnidium against one another resulted always in intermingling of the hyphae when the cultures met, indicating a uniformity in reaction for all spores from a given pycnidium. A similar lack of aversion resulted when representative colonies from the three pycnidia from a given culture were plated against one another, indicating that all the pycnidia from that culture were alike. Finally, plating the various cultures in their respective original positions resulted again in the designs originally observed in each plate group (fig. 2, *C*). This procedure was carried through three successive pycnidial generations with no changes in the behavior of any of the progenies, thereby demonstrating the stability of the aversion reactions of the strains through three pycnidial generations.

STABILITY FOLLOWING REISOLATION FROM INOCULATED CORN PLANTS

Cultures from the third pycnidial generation were used for field inoculations to study possible changes in reactions following passage through the host. The seven cultures illustrated in figure 2, *C*, were used in this experiment. A spore suspension from each of the cultures was injected with a hypodermic needle into the tips of five ears in each of three inbred lines of dent corn. Platings of the reisolated cultures against the original stock cultures proved that no changes had occurred in the reactions of any of the strains. When the isolates again were combined on plates in their original relative positions, the same design again was obtained (fig. 2, *D*), thus demonstrating the stability of the strains through the pathogenic phase of their life cycles.

NUMBER AND DISTRIBUTION OF STRAINS

Some information on the extent and distribution of strains of *Diplodia zeae* was obtained from experiments in which randomly selected cultures were paired on plates in all possible combinations. Twenty-five cultures of *D. zeae*, isolated from 25 ears of corn collected from 1 field near Bloomington, Ill., were used for a so-called local test. The results from the platings showed 1 strain to occur 3 times, 2 others to occur twice each, and 18 to occur but once, thus making a total of 21 strains in this random group of 25 cultures. For a regional test 25 cultures of *D. zeae* were obtained from as many widely separated points throughout the Corn Belt. In this experiment only two of the cultures proved to be of the same strain. Observational evidence from plating many hundreds of colonies of *D. zeae* from damaged kernels in terminal market samples of corn indicates further the existence of a multiplicity of strains. Aversion between adjacent colonies has usually been the rule, intermingling being relatively rare.

INOCULATIONS WITH MIXTURES OF STRAINS

Inoculation experiments involving mixtures of strains of *Diplodia zeae* suggested two problems of particular interest: (1) Would aversion function in the host tissues to such an extent that one strain would inhibit the further development of the others and thus singly overrun the host? (2) If this should prove to be true, would it be possible to use such mixtures for determining the relative pathogenicity of strains? In field experimental work, a mixture of several cultures frequently has been used on the assumption that this provided a safeguard against the use of an inferior culture.

EXPERIMENTS IN 1934

Three strains of *Diplodia zeae* isolated from rotted corn kernels from Maryland, Ohio, and Illinois were used in a mixed-strain experiment conducted at Madison, Wis., in 1934. In future references these strains are called nos. 150, 26, and 73, respectively. Two inbred lines of corn, namely, R4, which is resistant to ear infections of *D. zeae*, and Lan, which is susceptible, were inoculated by hypodermic injections of spore suspension into the tips of the ears while in the milk stage of development. Care was taken that the cultures were of the same age and that the inocula used were of approximately equal spore concentrations. Inoculations first were made with single cultures to test for pathogenic differences between the fungus strains, after which equal volumes of the remaining inocula were combined and used for the mixed-strain inoculations. The inoculated ears were harvested about 6 weeks later. It was apparent that the extent of rotting that had developed in the ears was no more severe when the mixture of strains was used than when the strains were used separately. Nor were any decided differences in pathogenicity evident between the three strains used. These relationships held in both the resistant and susceptible inbred lines.

The identity of the fungus strains recovered from the rotted ears was established by means of a series of platings that involved, finally, the pairings of representative cultures from each ear against the stock cultures of the strains used in the experiment. Five kernels, taken spirally from each of the rotted ears, were used as a basis for the determination of the strain or strains in the ear. The results from the identification of the reisolated cultures are given in table 1.

TABLE 1.—*Strains of Diplodia zeae recovered from ears of corn inoculated with single strains and with a mixture of these strains at Madison, Wis., 1934*

Strains used for inoculum	Inbred [1] lines inoculated	Ears inoculated	Inoculated ears from which indicated strains were recovered		
			No. 150	No. 73	No. 26
Single strains:		*Number*			
No. 150	R4	15	15		
	Lan	15	15		
No. 73	R4	15		15	
	Lan	15		15	
No. 26	R4	15			15
	Lan	15			15
Mixed strains:					
Nos. 150, 73, and 26	R4	15			14
	Lan	14		[2] 1	14

[1] R4 is resistant and Lan is susceptible to diplodia ear rot.
[2] Tip kernels of 1 ear plated strain 73; the remainder of the ear plated strain 26.

It will be observed from table 1 that the strain recovered from each of the single-strain inoculations was the same as that used in the inoculum. This was to be expected in view of the stability of the aversion reactions of strains of *Diplodia zeae* as established in the earlier experiments. The data also show that in the mixed-strain inoculations, with one exception, strain no. 26 was the only one reisolated, and apparently it had completely inhibited the development of strains nos. 150 and 73, both in the resistant and in the susceptible inbred line of corn.

EXPERIMENTS IN 1935

In view of the consistency with which strain no. 26 inhibited the others in the mixed inoculations in 1934, it was decided to repeat the experiment with certain modifications the following season. As no differentials in reaction between inbred lines and fungus strains were obtained in 1934, it was decided to use but one inbred, Wis. R3, in 1935, and to concentrate on a somewhat more elaborate series of strain mixtures.

A strain of *Diplodia macrospora* was added to the three strains of *D. zeae* previously used for observations on interspecific aversion. In addition to the single-strain inoculations, the following mixtures were used: *D. zeae*, nos. 150 and 73; 150 and 26; 73 and 26; 150, 73, and 26; and *D. macrospora* and *D. zeae* nos. 150, 73, and 26. The methods for inoculation and for identification of cultures recovered were similar to those of the previous season. The results from the experiments are given in table 2.

TABLE 2.—*Strains of Diplodia zeae and D. macrospora recovered from ears of corn of inbred line Wis. R3 inoculated with single strains and various mixtures of these strains and species, at Madison, Wis., 1935*

Species and strains used as inoculum	Ears inoculated	Inoculated ears from which the indicated strains were recovered				
		D. macrospora	*D. zeae* no. 150	*D. zeae* no. 73	*D. zeae* no. 26	Mixture of strains
Single strains:	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
D. macrospora	14	[1] 12	0	0	0	0
D. zeae no. 150	14	0	14	0	0	0
D. zeae no. 73	15	0	0	15	0	0
D. zeae no. 26	11	0	0	0	11	0
Mixed strains:						
D. zeae nos. 150 and 73	15	0	0	15	0	0
D. zeae nos. 150 and 26	13	0	0	0	13	0
D. zeae nos. 73 and 26	15	0	0	1	14	0
D. zeae nos. 150, 73, and 26	36	0	0	0	28	[2] 8
D. macrospora and *D. zeae* nos. 150, 73, and 26	33	0	2	1	24	[3] 6

[1] Of 14 ears inoculated 12 developed ear rot.

[2] Strain no. 26 predominated in 6 of the 8 ears from which more than 1 strain was isolated. Strain no. 73 predominated in 2 of the 8 ears. Strain no. 150 was isolated from tip kernels in only 1 ear.

[3] *D. zeae* no. 73 predominated in 4 of the 6 ears from which more than 1 strain was isolated. *D. zeae* no. 150 predominated in 2 of the 6 ears. *D. zeae* no. 26 was isolated from all 6 of the ears. *D. zeae* nos. 73, 150, and 26 were all isolated from 1 ear. *D. macrospora* was absent in all ears.

Except for *Diplodia macrospora*, which appeared decidedly less pathogenic under the conditions of this experiment, no differences in extent of ear rot were evident in any of the inoculations, either in the comparisons between strains or between the various mixtures of

strains. The data in the table show that the cultures recovered from the single-strain inoculations were the same as those used in the inoculum, again demonstrating stability following passage through the host.

Of the three strains of *Diplodia zeae*, no. 73 alone was recovered from the 15 ears inoculated with a mixture of nos. 73 and 150. Apparently it had completely inhibited the development of the latter. In a similar manner strain no. 26 completely inhibited the development of strain no. 150 in all of the 13 ears inoculated with a mixture of the two strains. Strain no. 26 inhibited strain no. 73 in 14 out of 15 ears inoculated with this mixture and was itself inhibited by strain no. 73 in the 1 remaining ear. Thus a definite sequence in inhibitory effects occurred among these three strains, no. 73 inhibiting no. 150, and no. 26 inhibiting both no. 73 and no. 150.

Strain no. 26 in most instances maintained its inhibitory powers in the mixture involving all three strains. It alone was recovered from 28 of the 36 ears inoculated with this mixture. In addition to no. 26, other strains also were recovered from each of the remaining eight ears of this group. Strain no. 26 predominated on six of these ears (it alone was plated in the region between the middle and the butt of the ear); strain no. 73 predominated on two of the ears; and strain no. 150 was recovered from tip kernels of only one ear.

The results from the mixture involving the three strains of *Diplodia zeae* and one strain of *D. macrospora* again showed *D. zeae* no. 26 to be the dominating one. It alone was recovered from 24 of the 33 ears in this group. *D. zeae* no. 150 was the only one recovered from two ears, and *D. zeae* no. 73 was the only one recovered from one ear. *D. zeae* no. 26 also was recovered from each of the remaining six ears that yielded more than one strain of the fungus. *D. zeae* no. 73 predominated on four of these ears, and *D. zeae* no. 150 predominated on the remaining two ears. In no case was *D. macrospora* recovered.

These experiments demonstrated that *Diplodia zeae* no. 26 was able to manifest its inhibitory powers, in the various mixtures of strains used, in two successive seasons and in three inbred lines of corn.

DISCUSSION

The occurrence of intraspecific aversion in *Diplodia* represents an exception to the general rule as stated by Porter (*7*), i. e., that antagonism between fungi becomes more marked as the phylogenetic relationships are widened. It is beyond the scope of the present paper to discuss the hypotheses that have been advanced to explain aversion, but it is desirable to point out an important and basic difference between aversion in *Diplodia* and a phenomenon sometimes observed within specific lines in other fungi. Accepting Brown's definition (*2, p. 106*) of staling as the effect of "those metabolic products of the organism responsible for slowing down or stopping its growth", in the opinion of the author it becomes apparent that aversion in *Diplodia* is not due to staling products. Aversion in *Diplodia* results from the interaction between two physiologically different strains. In some other genera an entirely different situation exists, which might be confused with intraspecific aversion. In *Chaetomium* sp., for example, a very distinct inhibition of growth occurs when two colonies approach each other in culture. However, an identical reaction is observed

when a given culture is plated against itself. This, it seems, is either a nutritional response, or, more probably, a reaction closely allied to Brown's conception of staling. In either case it does not establish the existence of distinct physiological differences such as appear to exist in the strains of *Diplodia*.

In the literature dealing with intraspecific aversion the aspect of the problem studied most intensively concerns the relationship between the phenomenon and sex. Among studies on the inheritance of the phenomenon and its complex sexual relationships in Ascomycetes and in Hymenomycetes are papers by Cayley (*3*, *4*), Vandendries (*8*, *9*), and Vandendries and Brodie (*10*).

Little of the previous work on aversion is directly analogous with the studies reported herein with *Diplodia*. The fact that like meets like with intermingling of the mycelia and that aversion results when unlike strains meet has been reported by Cayley (*3*) in a homothallic form of *Diaporthe perniciosa*, by Nakata (*6*) in *Sclerotium rolfsii*, and by Mounce (*5*) in *Fomes pinicola*. A case in which unlike strains sometimes intermingle is described by Cayley (*4*), and this she believes to be a heterothallic form of *Diaporthe*. In this instance mycelia which meet can segregate into averting and nonaverting strains in later perithecial generations.

The uniformity in reaction of all monosporic mycelia from individual pycnidia in *Diplodia zeae* is in agreement with Cayley's (*3*) results with *Diaporthe*.

The multiplicity of strains found in *Diplodia zeae* agrees generally with studies on this aspect by Cayley (*3*), Mounce (*5*), and Nakata (*6*), with *Diaporthe perniciosa*, *Fomes pinicola*, and *Sclerotium rolfsii*, respectively.

No previous accounts of mixed-strain inoculations are known to the writer. Cayley (*4*) raised the question whether the aversion reaction might possibly be inhibited in host tissues owing to absorption of the toxins secreted by the fungus. A case of aversion between colonies of *Corticium calceum* on a piece of pine wood was recently described by Brodie (*1*). He believed this to be the first reported case of the occurrence of the phenomenon in nature.

The results from the mixed-strain inoculations with *Diplodia* indicate that the aversion reaction functions within the host. The consistency with which a strain eventually gets the upper hand and rots the major portion of the ear can hardly be explained on any other basis. The writer does not recall having plated more than one strain of *D. zeae* from naturally infected ears, except possibly where separate infections had occurred, one at the tip and another at the butt end of the ear.

No conclusions could be drawn from the mixed-strain experiments regarding the relationship between ability to inhibit and degree of pathogenicity. Despite the definite sequence in inhibitory powers manifested among the strains in the mixed inoculations, no appreciable differences in extent of ear rot were apparent when the strains were used singly in inoculations. It is quite conceivable, however, that an improved technique for comparing relative pathogenicity might reveal differences not apparent in these experiments. On the other hand, it is possible that physiological studies on the fungus strains might show differences in rates of spore germination and subsequent growth which might be correlated with their so-called inhibitory powers.

The results from the inoculations with mixtures of strains indicate that, for practical purposes, the use of mixed cultures accomplishes little beyond the diluting of the dominating strain used. The method does provide a technique for the selection of a very dominating strain for inoculations in experimental work.

The many strains of *Diplodia zeae*, each of which apparently remains constant or stable for its aversion character, raises a question as to the origin of all these strains. If the capacity of strains to inhibit others is an attribute of *Diplodia* strains generally, should not the strains in a given region have become limited through natural selection to a relatively small number? This situation seemingly does not exist. The evidence reported herein indicates that the differences among the strains are genetic and suggest the possible existence in this species of a sexual stage not yet described.

SUMMARY

The phenomenon of intraspecific aversion, infrequently reported in fungi, has been found in *Diplodia zeae* (Schw.) Lev. and *D. macrospora* Earle, and interspecific aversion has been found between these two species.

The stability of the particular aversion reactions of the different strains of *Diplodia zeae* was maintained, after successive mycelial propagations, through three successive pycnidial generations in culture and through inoculation into ears of corn and subsequent reisolation.

The number of strains of *Diplodia zeae* apparently is very large. Twenty-one different strains were obtained from 25 cultures isolated from as many ears of corn collected in one field near Bloomington, Ill. Among 25 isolates from widely separated points throughout the Corn Belt, 24 strains were obtained.

Field experiments involving inoculations with single strains and with mixtures of strains were conducted at Madison, Wis., in 1934 and 1935. Inoculations with various combinations of three strains of *Diplodia zeae* and a strain of *D. macrospora* were made in three inbred lines of corn. Identification of the strains recovered from the subsequently rotted ears proved that inhibitory effects had occurred so that usually only one strain of the fungus could be reisolated from an ear. It was also found that a definite sequence existed in the inhibitory powers of strains upon one another. So far as tried, *D. zeae* predominated over *D. macrospora*.

No conclusions could be drawn regarding the relationship between the capacity of strains for inhibiting the development of others and their degrees of pathogenicity.

LITERATURE CITED

(1) Brodie, H. J.
1935. The occurrence in nature of mutual aversion between mycelia of hymenomycetous fungi. Canad. Jour. Research 13: 187–189, illus.

(2) Brown, W.
1923. Experiments on the growth of fungi on culture media. Ann. Bot. [London] 37: [105]–129, illus.

(3) Cayley, D. M.
1923. The phenomenon of mutual aversion between mono-spore mycelia of the same fungus (diaporthe perniciosa, marchal), wth a discussion on sex heterothallism in fungi. Jour. Genetics 13: [353]–370, illus.

(4) CAYLEY, D. M.
1931. THE INHERITANCE OF THE CAPACITY FOR SHOWING MUTUAL AVERSION BETWEEN MONO-SPORE MYCELIA OF DIAPORTHE PERNICIOSA (MARCHAL). Jour. Genetics 24: 1–63, illus.

(5) MOUNCE, I.
1929. THE BIOLOGY OF FOMES PINICOLA (S. W.) COOKE. STUDIES IN FOREST PATHOLOGY. Canada Dept. Agr. Bull. (n. s.) 111, 74 pp., illus.

(6) NAKATA, K.
1925. STUDIES IN SCLEROTIUM ROLFSII SACC. PART 1. THE PHENOMENON OF AVERSION AND ITS RELATION TO BIOLOGIC FORMS OF THE FUNGUS. Bull. Sci. Fakult. Terkult. Kjusu Imp. Univ. 1: 177–190.

(7) PORTER, C. L.
1924. CONCERNING THE CHARACTERS OF CERTAIN FUNGI AS EXHIBITED BY THER GROWTH IN THE PRESENCE OF OTHER FUNGI. Amer. Jour. Bot. 11: 168–188, illus.

(8) VANDENDRIES, R.
1932. LA TÉTRAPOLARITÉ SEXUELLE DE PLEUROTUS COLUMBINUS. DÉMONSTRATION PHOTOGRAPHIQUE D'UN TABLEAU DE CROISEMENTS. Cellule 41: [267]–277, illus.

(9) ———
1933. DE LA VALEUR DU BARRAGE SEXUAL COMME CRITÉRIUM DANS L'ANALYSE D'UNE SPORÉE TÉTRAPOLAIRE DE BASIDIOMYCÈTE: PLEUROTUS OSTREATUS. Genetica 15: 202–212.

(10) ——— and BRODIE, H. J.
1933. NOUVELLES INVESTIGATIONS DANS LE DOMAINE DE LA SEXUALITÉ DES BASIDIOMYCÈTES ET ÉTUDE EXPÉRIMENTALE DES BARRAGES SEXUELS. Cellule 42: [165]–209, illus.

THE EFFECT OF STORAGE UPON THE VITAMIN A CONTENT OF ALFALFA HAY [1]

By MARGARET CAMMACK SMITH

Nutrition chemist, Arizona Agricultural Experiment Station

INTRODUCTION

In a previous paper from this station [2] the vitamin A content of alfalfa was shown to be affected by exposure of the leaves to sunshine during the curing process. At the time of that investigation it was observed that the vitamin A value of the alfalfa leaves had to be determined as soon as the hay reached the laboratory if consistent results were to be obtained. When the vitamin tests were repeated on the same samples of hay later in the year, the vitamin A values were lower. This suggested the advisability of studying the effect of storage upon the vitamin A content of alfalfa.

The studies of Hauge and Aitkenhead [3] indicated that enzymes are responsible for the destruction of vitamin A which occurs during the field-curing process, and it seemed logical to suppose that this destructive action might continue, though perhaps more slowly, in alfalfa stored under commercial conditions after baling. In 1930 Quinn, Hartley, and Derow [4] reported that spinach when finely ground and kept in a mason jar in the laboratory lost approximately 70 percent of its vitamin A during a 12- to 15-month storage period.

Guilbert [5] has recently made a study of the factors that cause variations in the carotene content of alfalfa hay and meals. On the assumption that variations in the carotene content are a criterion of variations in the vitamin content, he interprets his findings in terms of the effect of these factors upon the vitamin A value of alfalfa. Guilbert finds that temperature is the major factor causing a loss of carotene in alfalfa during storage and that the rate of loss is roughly doubled for each 10° C. rise in temperature.

This paper briefly reports the effect of storage of alfalfa hay in Arizona upon its vitamin A content as determined by biological assay.

EXPERIMENTAL MATERIALS AND METHODS

The alfalfa used was of the Hairy Peruvian variety produced at the Salt River Valley Experiment Farm, Mesa, Ariz. The hay was cut with a mower and left in the windrows until wilted. It was then raked, shocked in small cocks, cured, and baled. Immediately after baling, it was stored in a hay shed on the farm. At intervals of 3

[1] Received for publication June 30, 1936; issued Nov. 1, 1936.

[2] SMITH, M. C., and BRIGGS, I. A. THE VITAMIN CONTENT OF ALFALFA AS AFFECTED BY EXPOSURE TO SUNSHINE IN THE CURING PROCESS. Jour. Agr. Research 46: 229–234. 1933.

[3] HAUGE, S. M., and AITKENHEAD, W. THE EFFECT OF ARTIFICIAL DRYING UPON THE VITAMIN A CONTENT OF ALFALFA. Jour. Biol. Chem. 93: 657–665, illus. 1931.

[4] QUINN, E. G., HARTLEY, J. A., and DEROW, M. A. SOME OBSERVATIONS ON THE BEHAVIOR OF VITAMIN A IN OR FROM PRIMARY SOURCES. Jour. Biol. Chem. 89: 657–663. 1930.

[5] GUILBERT, H. R. FACTORS AFFECTING THE CAROTENE CONTENT OF ALFALFA HAY AND MEAL. Jour. Nutrition 10: 45–62, illus. 1935.

months, one bale of alfalfa from the same cutting and curing was sent to the nutrition laboratory of the University of Arizona at Tucson. In the laboratory the leaves of a representative sample of the bale were carefully separated by hand from the stems and petioles so as to obtain a uniform sample. This material was kept in covered glass jars during the conduct of the test. The vitamin A measurement was begun at once with animals that had been prepared in advance for the test.

The first study was made on alfalfa baled August 9, 1932, and a second was made the following year with the 1933 crop of alfalfa baled August 11.

In the biological assay of the vitamin A content of the alfalfa, the standard method of Sherman and Munsell, as modified somewhat by Sherman and Burtis,[6] was used. The method consisted essentially in feeding graded portions of alfalfa as the sole source of vitamin A to rats which had been maintained upon a vitamin A-deficient ration until their body stores of the vitamin had become exhausted. The growth response to the alfalfa supplement given daily (6 days) during an 8-week experimental period was used as the criterion of the amount of vitamin A in the alfalfa fed. Representative animals from each litter were kept as controls and other details of the test were conducted in the usual manner.

EXPERIMENTAL RESULTS

The growth responses of the rats to the feeding of weighed amounts of alfalfa varying from 0.005 to 0.050 gms are presented in summarized form in table 1. It may be noted that the results for the crops of 1932 and 1933 are quite similar.

When the vitamin A test was begun in August upon the freshly baled alfalfa, a high vitamin A potency was observed. Five milligrams of the alfalfa leaves were found to induce a gain of 41 g during the 8-week test. This is considerably greater than the unit rate of gain as defined by Sherman of 25 g in 8 weeks. When the test was repeated in November on another bale of hay which had been stored as previously described for 3 months, the growth response to the same levels of alfalfa supplement was strikingly different. In November twice as much alfalfa was required to induce approximately the same rate of gain.

The vitamin A content determined in February of the alfalfa which had been stored on the farm since August (a 6-month period) was far lower than that of the freshly baled hay in August, but it was of practically the same order as that obtained in November. The test made at this time showed a gain of approximately 40+ g resulting from the daily supplement of 10 mg of alfalfa for 8 weeks, or an approximate unit rate of gain induced by 7.5 mg of this alfalfa. Thus, no further destruction of vitamin A during the storage of alfalfa for the 3 months, November to February, was apparent.

[6] SHERMAN, H. C., and BURTIS, P. FACTORS AFFECTING THE ACCURACY OF THE QUANTITATIVE DETERMINATION OF VITAMIN A. Jour. Biol. Chem. 78: 671–680. 1928.

TABLE 1.—*Summarized results of feeding alfalfa stored for different lengths of time to albino rats as the sole source of vitamin A*

Length of storage period (months)	1932 crop				1933 crop			
	Amount of alfalfa leaves fed daily	Rats used in test	Average gain or loss in weight	Survival in test period	Amount of alfalfa leaves fed daily	Rats used in test	Average gain or loss in weight	Survival in test period
	Grams	*Number*	*Grams*	*Days*	*Grams*	*Number*	*Grams*	*Days*
0	0	4		29	0	4		30
	.005	6	+41		.005	8	+45	
	.0075	6	+63		.0075	9	+57	
	.01	6	+71		.01	9	+68	
3	.0	6		37	.0	8		25
	.005	10	+13		.005	11		53
	.0075	11	+26		.0075	9	+19	
	.01	7	+38		.01	8	+40	
6	.0	6		27	.0	8		23
	.005	7		45	.005	8		57
	.0075	5	+23		.0075	8	+29	
	.01	5	+42		.01	8	+45	
9	.0	8		27	.0	8		24
	.005	5		30	.005	8		37
	.0075	2		36	.0075	10		42
	.01	8	+8		.01	8		50
12	.0	8		32	.0	8		30
	.005	2		21	.005	6		36
	.0075	2		38	.0075	6		47
	.01	4	+15		.01	8	+21	
	.02	6	+41		.02	8	+45	
	.03	4	+58		.03	6	+65	
	.05	10	+77		.05	4	+83	

When the measurement was repeated 3 months later in May on alfalfa which had been stored for 9 months, the results showed an even greater loss in vitamin A potency. Unfortunately, in this case the highest level at which the alfalfa was fed, 10 mg daily, was not sufficient to induce unit rate of gain. Most of the animals did not survive the test period, and on autopsy all showed a condition typical of vitamin A deficiency. Storage of the alfalfa until August, a period of 12 months, resulted in a product which was very inferior to the freshly baled hay. It required the daily feeding of 20 mg of this hay to promote a gain of approximately 40 g in the test animals in the 8-week period, a rate of gain which resulted from the daily feeding of only 5 mg of the hay which was tested before storage. Thus it appears that hay stored in Arizona during the 3 months, August, September, and October, undergoes marked deterioration, the destruction of its vitamin A content amounting to 50 percent. Further storage during November, December, and January resulted in no further measurable destruction. The alfalfa tested after 6 months' storage was also found to contain about 50 percent less vitamin A than the original freshly baled alfalfa. When, however, the hay was stored for an even longer time, there was a further loss in vitamin A. From February to August there was another loss of 50 percent of the vitamin A, which was found in the alfalfa stored for 6 months, or until February. This meant that by the following August the alfalfa leaves contained but 25 percent as much vitamin A as the original material baled 12 months previously.

In general, these findings agree with the work of Guilbert, who found that the rate of carotene destruction in alfalfa varied with the season of the year. He reported a decrease of 9 percent in carotene content in chopped sun-cured alfalfa hay after storage in a dark loft in Davis, Calif., from November to April, and a further decrease of 30 percent in hay stored from April to August. His belief that the temperature of the environment is a major factor in the rate of carotene or vitamin A destruction offers a satisfactory explanation of the findings reported herein relative to the rate of destruction of vitamin A under storage conditions in Arizona. During the first 3-month storage period in 1932 the mean maximum temperature ranged from 101° to 83° F. as compared with a mean maximum temperature range of 77° to 58° in the following 3 months. The temperature in Mesa rises sharply in the spring, and May, June, and July are hot months, with a mean maximum ranging from 84° to 103°. The rate of destruction of vitamin A is thus seen to be rapid in hot weather, and slow or at a standstill during the winter months of storage.

CONCLUSIONS

Leaves of baled alfalfa stored from August to November in a hay barn in Mesa, Ariz., contained 50 percent less vitamin A than the leaves of freshly baled alfalfa, as determined by biological assay. No further destruction of vitamin A occurred during subsequent storage in the cold months of November, December, and January. With rising temperature in the spring, the destruction of vitamin A was again stimulated. After 12 months in storage the alfalfa contained only 25 percent as much vitamin A as had been found in the fresh alfalfa.

PHYTOPHTHORA ROOT ROT OF CAULIFLOWER[1]

By C. M. TOMPKINS, *assistant plant pathologist, California Agricultural Experiment Station;* C. M. TUCKER, *plant pathologist, Missouri Agricultural Experiment Station;* and M. W. GARDNER, *plant pathologist, California Agricultural Experiment Station*[2]

INTRODUCTION

A destructive root rot of cauliflower (*Brassica oleracea* L. var. *botrytris* L.) occurs during the winter in certain coastal regions of California (*10*),[3] mainly in low or poorly drained spots in heavy soils that are subject to waterlogging during irrigation or rainy periods. Considerable losses as a result of this disease have been observed in all varieties in the San Francisco Bay section. The disease has also been found near Colma, Half Moon Bay, and Salinas. Other naturally infected hosts are cabbage (*B. oleracea* L. var. *capitata* L.), Brussels sprouts (*B. oleracea* L. var. *gemmifera* DC.), and hybrid cineraria (*Senecio cruentus* DC.) grown in a lath house at Salinas. J. B. Kendrick isolated the fungus from diseased stock or gilliflower (*Mathiola incana* R. Br. var. *annua* Voss) collected in Solano County in January 1934.

The disease is caused by *Phytophthora megasperma* Drechsler.

SYMPTOMS

The disease is characterized by a reddish discoloration of the older leaves followed by a sudden wilting of all the older leaves, which fall prostrate to the ground (fig. 1), leaving the head or curd exposed. The curd is not noticeably discolored but becomes tough and rubbery and is unmarketable. Plants of all ages are susceptible.

The lower end of the taproot, often along with the underground part of the stem, is badly rotted (fig. 2), and infected plants may be pulled from the soil with little effort. The cortex of the taproot and lateral roots is softened and water-soaked (fig. 2, *B*), usually sloughs off, and remains in the soil when the plant is pulled. The woody cylinder or stele is discolored and often frayed at the lower end (fig. 2, *A*). About the upper edge of the diseased part of the root there may be callus formation in the cortex (fig. 2, *A*), and numerous adventitious roots are sometimes produced (fig. 2, *C*). The upper margin of the diseased tissues of the cortex, stele, and pith is marked by a blackened zone. The infected pith is often collapsed, with resultant cavities.

In earlier stages of infection, lateral cankers are found in the cortex of the root, but in plants that show leaf symptoms the entire root and part of the underground stem are usually involved. Externally, the

[1] Received for publication Apr. 14, 1936; issued Nov. 1, 1936. Joint contribution from the Division of Plant Pathology, California Agricultural Experiment Station, and the Department of Botany, Missouri Agricultural Experiment Station.

[2] The writers express their grateful appreciation to Prof. Ralph E. Smith, of the California Station, for advice and assistance. The Division of Agronomy, University of California, generously supplied greenhouse and outdoor facilities for the inoculation tests.

[3] Reference is made by number (italic) to Literature Cited, p. 692.

diseased tissues may be Isabella color, sepia, or black; internally, ivory yellow to sepia of Ridgway (*9*).

Typically, the reddish discoloration of the older leaves consists of a broad, marginal band with interveinal extensions toward the midrib, the upper surface being Pompeian red to neutral red, the lower surface Vernonia purple.

THE CAUSAL FUNGUS, PHYTOPHTHORA MEGASPERMA

The fungus is readily isolated by planting tissue fragments taken from the margin of the lesion in malt-extract agar (*7*). The same fungus was obtained from diseased cauliflower plants collected in

FIGURE 1.—Phytophthora root rot of cauliflower in a low area in the field, February 14, 1934. The wilting is preceded by a reddish discoloration of the older leaves.

various localities and from cabbage, Brussels sprouts, stocks, and cineraria hybrids. It is found only in the root system and underground portions of the stem.

Freehand cross and longitudinal sections of naturally infected roots were stained with fast green and Magdala red. The fungus is intercellular. Drechsler (*4*) observed the mycelium of *P. megasperma* to be intercellular, and Dowson (*3*) found an abundant intercellular mycelium in decaying carrots.

The fungus grows rapidly on the ordinary culture media. On oatmeal agar it produces short, tufted aerial growth which gives the surface a somewhat mealy appearance. Sexual spores develop early and very abundantly on this medium. Transfers of isolates of *Phytophthora megasperma* from hollyhock, the type culture of Drechsler (*4*), stock, cabbage, cauliflower, cineraria, and of a culture (host not specified) which was sent to the Centraalbureau voor Schimmelcultures,

Baarn, Netherlands, by Ashby, behave similarly in cultural characters and spore production. Oogonia and oospores from oatmeal-agar cultures vary somewhat in size, as shown in table 1.

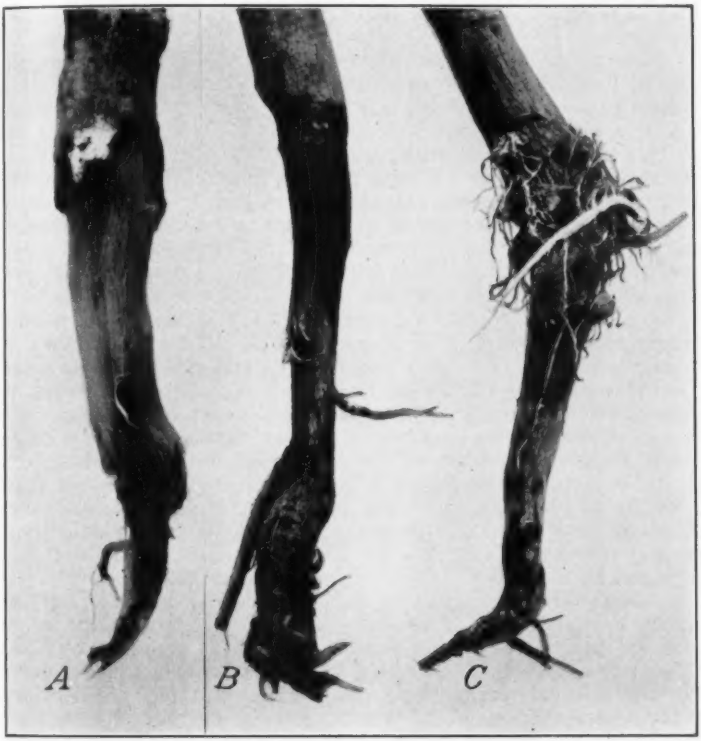


FIGURE 2.—Phytophthora root rot of cauliflower: *A*, Shredding of woody cylinder and callus at lower edge of healthy cortex; *B*, dark discoloration of rotted cortex; *C*, formation of adventitious roots above rotted portion of taproot.

TABLE 1.—*Diameter of oogonia and oospores of Phytophthora megasperma from various sources*

Source	Oogonial diameters		Oospore diameters	
	Limits	Average	Limits	Average
	Microns	*Microns*	*Microns*	*Microns*
Cauliflower:				
1	32–43.2	38.2	29.2–36.4	33.4
2	32–46.0	38.4	26 –40	33.2
Stock:				
1	30–48	42.8	26 –45.6	39.6
2	32–50	42.0	26 –44	37.6
Cabbage	32–43.6	38.0	28 –40	33.2
Hollyhock	36–52	42.6	32.8–45.2	37.2
Ashby culture	32–47.2	41.6	28 –41.6	36.0
Cineraria	31–41.7	35.9	29.7–38	33.1

The size of the oogonia and oospores is affected by temperature, smaller spores developing at temperatures near the maximum, and by the culture medium, those produced on moist corn meal being smaller than those produced on oatmeal agar. Oogonia and oospores produced in liquid media are generally slightly smaller than those produced on solid media. The size of the oogonia and oospores is, therefore, subject to variation. However, all observed are sufficiently larger than those of *Phytophthora cactorum* to permit ready separation of cultures of the two species. The oogonia of *P. megasperma* are thin-walled, usually spherical or subspherical, with a short, rather slender stalk (pl. 1), while those of *P. cactorum* are inflated in the region enclosing the oospore and taper to form a broad funnel-shaped stalk. The latter type often appears in cultures of *P. megasperma* but is not typical of the species. The oogonia of *P. syringae* and *P. hibernalis* resemble those of *P. cactorum*. The oospores of *P. megasperma* occur singly in the oogonia, almost filling them (pl. 1, *C–G*); the oospore wall is smooth, thick, lemon yellow to straw color. Germination was not observed.

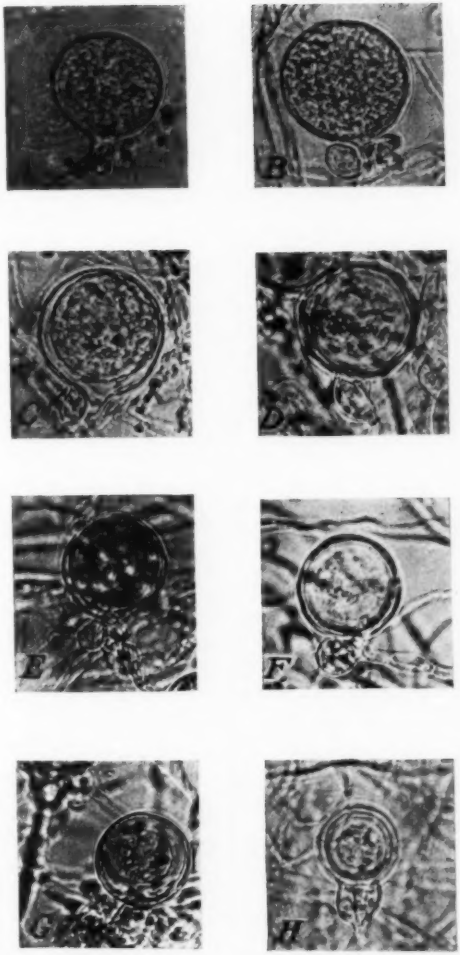
The antheridia of *Phytophthora megasperma* are preponderantly paragynous in type (pl. 1, *B–G*), yet variable proportions of them are amphigynous (pl. 1, *H*). The latter type is produced more frequently in liquid than on solid media, and in many cases the oogonia and oospores are smaller than those with paragynous antheridia. In all cultures examined the paragynous greatly outnumbered the amphigynous antheridia. None of the latter was found in oatmeal-agar cultures of some of the isolates. The antheridia may be borne on different or the same hyphae on which the oogonia develop; sometimes the antheridia develop on a short branch originating on the same hypha and only a few microns from the point of attachment of the stalk of the oogonium.

Sporangia do not develop on the solid media used, and but very scantily in liquids. A few appeared on hyphae grown 4 days in pea broth and transferred to sterile distilled water, as suggested by Rands (*8*) in 1922 and more recently by Leonian (*6*). They were obpyriform with a broad rounded base, nonpapillate, but with a thin refringent layer at the apical end, resembling those described by Drechsler (*4*). They measured 41.6 to 56 by 28 to 40 microns, averaging 49.4 by 33.6 microns. Zoospore development and discharge were observed. Attempts to induce sporangium development by flooding cultures on Difco and unfiltered corn-meal agar and lima-bean agar with sterile distilled water and by transferring hyphae from oatmeal-agar cultures to Petri's solution and a nonsterile soil suspension were not successful.

The temperature relations of the cauliflower fungus are similar to those of the other isolates of the species, as indicated by the results of plate tests in which Difco corn-meal agar was used.

Of the other species with paragynous antheridia *Phytophthora hibernalis* and *P. syringae* cannot tolerate the high temperatures at which *P. megasperma* grows well. The temperature relations of *P. cactorum* are similar to those of *P. megasperma*.

The species appears to be quite distinct, and is one of the most easily recognized because of its large sexual spores which are produced early and profusely. It was not included in the key for the identifica-



PHYTOPHTHORA MEGASPERMA DRESCHLER. × 465.

A, Young oogonium without antheridium. *B*, Unfertilized oogonium and antheridium. *C*, Fertilized oogonium with developing oospore. *D–G*, Oogonium with oospores in various stages of development, as indicated by the thickening of the walls of the oospores, the antheridia in *B–G* being paragynous. *H*, Oogonium with amphigynous antheridium.

tion of species published by Tucker (*11, p. 190*) in 1931, which may now be emended as follows:

B. Widely spreading growth on malt-extract agar and ordinary agar media after 6 days at 20° C.
1. Oogonia developing promptly (within 2 weeks) and abundantly on oatmeal agar or steamed corn meal.
Antheridia predominantly paragynous.
a. Growth on corn-meal agar after 4 days at 28° C.
(1) Average diameter of oospores (oatmeal-agar cultures) exceeding 30 microns. *P. megasperma.*
(2) Average diameter of oospores less than 30 microns. *P. cactorum.*
b. No growth on corn-meal agar after 4 days at 28° C. *P. syringae.*

TABLE 2.—*Diameter of mycelial growth of Phytophthora megasperma from various sources after exposure for 96 hours at different temperatures in plate tests*

Source	Diameter of mycelial growth after 96 hours at—		
	20° C.	25° C.	30° C.[1]
Cauliflower:	*Millimeters*	*Millimeters*	*Millimeters*
1	25	25	18
2		38	23
Stock:			
1	30	38	33
2		40	21
Cabbage		33	20
Hollyhock		43	8
Ashby culture		41	22
Cineraria		44	28

[1] No growth at 35° C.

Since the isolation and description of *Phytophthora megasperma* by Drechsler (*4*) in 1931 as the cause of a crown rot of the hollyhock in the District of Columbia, the species has been found widely distributed and attacking hosts not closely related. Cairns and Muskett (*1, 2*) in 1933 reported the isolation of the species from potato tubers affected by a pink rot in Northern Ireland, and its identification by Ashby. Fawcett (*5*) in 1933 reported the isolation of the same species, also identified by Ashby, from citrus roots in California. Dowson (*3*) obtained the fungus in Tasmania, from the roots of carrots growing in a reclaimed bog during an abnormally wet season, under conditions resembling those prevailing in fields where the species causes a root rot of cauliflower in California. It is worthy of note that Cairns and Muskett (*2*) emphasize the importance of soil drainage "sufficiently good to prevent the land from becoming unduly wet in times of heavy rainfall" as a means of preventing pink rot of potatoes caused, at least occasionally, by *P. megasperma.*

INOCULATIONS IN WATERLOGGED SOIL

Attempts to cause infection of potted cauliflower plants in the greenhouse, by adding inoculum to the soil and watering the plants heavily, were unsuccessful, even when the roots were wounded by needle pricks. Typical infection occurred on unwounded roots, how-

ever, when the pots were set in about 4 inches of water in buckets and incubated outdoors in tests made during February to May. The incubation period was about 3 weeks.

Inoculum was prepared by growing the fungus isolated from cauliflower on sterilized, moistened, cracked wheat and adding this to the soil in 6-inch pots, each containing a young cauliflower plant growing in autoclaved soil. On January 7, 12 potted plants of the February variety of cauliflower were slightly wounded by pricking the taproot with a sterilized needle. After the inoculum had been added to the soil, the pots were placed in buckets out of doors. The temperature varied from 14° to 20° C. On February 10, 10 of the inoculated plants showed typical wilting and reddening of the leaves, while the 6 noninoculated controls remained healthy. The fungus was reisolated from each of the 10 infected plants.

On March 8, 1935, 15 potted cauliflower plants with six leaves were inoculated without wounding the roots and placed in buckets of water along with five noninoculated controls. On March 26, all of the inoculated plants were infected, and the fungus was reisolated from 12 plants. The controls remained healthy in spite of the waterlogged condition of the soil. Another set of 15 plants was similarly inoculated on March 15, and on April 9 all were infected. The cortical tissues were completely softened and were readily sloughed off from the central cylinder. The fungus was reisolated from these plants. The five noninoculated controls remained healthy. Similar results were obtained in a set of 20 plants inoculated with the reisolated fungus on May 8. On May 24, 19 were infected and the fungus was reisolated from 18 roots. The five noninoculated controls were healthy.

EXPERIMENTAL HOST RANGE

By the use of the inoculation method that provided waterlogged soil, the fungus isolated from cauliflower proved to be pathogenic to stock (*Mathiola incana* var. *annua*) and wallflower (*Cheiranthus cheiri* L.).

On March 8, 1935, 15 potted stock plants in the six-leaf stage were inoculated without wounding the roots and placed in buckets of water along with 5 control pots. On April 19, 13 of the inoculated plants were infected, and the fungus was reisolated from each. The five controls remained healthy. A repetition of the test was made on May 10 with a culture reisolated from stock, and by June 2, 7 of the 10 inoculated stock plants were infected, and the fungus was reisolated. The four controls remained healthy.

On April 6, 20 potted wallflower plants were inoculated and placed in buckets of water along with 5 noninoculated control pots. On April 27 all of the inoculated plants were infected, and the fungus was reisolated from 16. The controls remained healthy. On June 7, 20 wallflower plants were inoculated with a culture reisolated from infected wallflower plants and on July 8, 12 were infected. The fungus was recovered in culture. The noninoculated controls remained healthy.

Ripe fruits of tomato *Lycopersicum esculentum* Mill. var. *commune* Bailey) were successfully infected. Inoculations were made by placing a small square of inoculum from an agar culture of the fungus

on the unbroken surface and keeping this moist with absorbent cotton under an inverted preparation dish. On January 15, 1936, four fruits were inoculated with a 6-day-old agar plate culture. On January 24, water-soaked lesions had formed measuring 5.0 by 7.0, 6.0 by 4.0, 5.0 by 4.0, and 5.0 by 5.0 cm. The two fruits used as controls remained healthy. The fungus was reisolated from all infected fruits. With the reisolated fungus, four tomato fruits were inoculated on January 30. Two fruits were infected on February 2 and the fungus was reisolated.

On March 5, 1936, eight cineraria plants in the six-leaf stage were inoculated without wounding the roots, and six noninoculated plants were used as controls. On March 22 five of the inoculated plants were infected and the fungus was reisolated from each, while the controls remained healthy.

Attempts to infect unwounded fruits of bell or sweet pepper (*Capsicum frutescens* L. var. *grossum* (Bailey)) and Early White Bush Scallop and Zucchine pumpkins (*Cucurbita pepo* L.), potato tubers (*Solanum tuberosum* L.), and roots of garden beet (*Beta vulgaris* L.), turnip (*Brassica rapa* L.), hollyhock (*Althea rosea* Cav.), cotton (*Gossypium hirsutum* L. var. Acala), Chinese hibiscus (*Hibiscus rosa-sinensis* L.), and carrot (*Daucus carota* L. var. *sativa* DC.) were unsuccessful. Dowson (*3*), using an isolation from rotted carrots, was unable to secure infection through unwounded surfaces. Potato tubers inoculated by placing mycelium and oospores in a small slit about 4 mm deep were invaded by the isolate from cineraria and by Ashby's culture. The type of infection was similar to that resulting from infection by numerous species of *Phytophthora*, the infected tissues becoming pink on exposure to the air. The isolates from stock, cabbage, hollyhock, and cauliflower did not cause infection. Similarly, inoculated apple fruits were infected by all isolates, with the development of a light brown, mealy type of decay.

SUMMARY

A root rot of cauliflower, caused by *Phytophthora megasperma* Drechsler, is responsible for losses in the winter cauliflower crop in the coastal districts of California. The disease occurs only where the soil has become waterlogged.

The disease also occurs on cabbage, Brussels sprouts, cineraria, and stock.

The outer leaves of infected cauliflower plants show a reddish discoloration and later the plants wilt rather suddenly. Affected plants are easily pulled, and the basal end of the taproot is found to be rotted to such an extent that the cortex usually sloughs off.

The cauliflower fungus is described and compared with isolates from certain other hosts.

Infection was obtained by adding the fungus to the soil of potted cauliflower plants and incubating them outdoors in buckets containing water about 4 inches deep. The incubation period was about 3 weeks. Infection was also obtained on stock, cineraria, and wallflower plants and on ripe tomato fruits. Attempts to infect certain other unwounded vegetables were unsuccessful.

LITERATURE CITED

(1) Cairns, H., and Muskett, A. E.
1933. phytophthora megasperma causing pink rot of the potato. Nature [London] 131: 277.
(2) ——— and Muskett, A. E.
1933. pink rot of the potato. Ann. Appl. Biol. 20: 381–403, illus.
(3) Dowson, W. J.
1934. phytophthora megasperma drechsler in tasmania. Brit. Mycol. Soc. Trans. 19: 89–90.
(4) Drechsler, C.
1931. a crown-rot of hollyhocks caused by phytophthora megasperma n. sp. Jour. Wash. Acad. Sci. 21: 513–526, illus.
(5) Fawcett, H. S.
1933. new locations for phytophtora citrophthora and p. hibernalis on citrus. Phytopathology 23: 667–669.
(6) Leonian, L. H.
1934. identification of phytophthora species. W. Va. Agr. Expt. Sta. Bull. 262, 36 pp., illus.
(7) Mehrlich, F. P.
1934. medium for growth of pythiaceous fungi. (Phytopath. Note) Phytopathology 24: 1127–1128.
(8) Rands, R. D.
1922. streepkanker van kaneel, veroorzaakt door phytophthora cinnamomi n. sp. Dept. Landb., Nijv. en Handel [Dutch East Indies] Meded. Inst. Plantenziekten Afd. 54, 53 pp., illus. [In Dutch, with English summary.]
(9) Ridgway, R.
1912. color standards and color nomenclature. 43 pp., illus. Washington, D. C.
(10) Tompkins, C. M., Tucker, C. M., and Gardner, M. W.
1935. a phytophthora root rot of cauliflower. (Abstract) Phytopathology 25: 893–894.
(11) Tucker, C. M.
1931. taxonomy of the genus phytophthora de bary. Mo. Agr. Expt. Sta. Research Bull. 153, 208 pp., illus.

A SUBIRRIGATION METHOD OF SUPPLYING NUTRIENT SOLUTIONS TO PLANTS GROWING UNDER COMMERCIAL AND EXPERIMENTAL CONDITIONS [1]

By ROBERT B. WITHROW, *research assistant in horticulture*, and J. P. BIEBEL, *graduate assistant in horticulture, Purdue University Agricultural Experiment Station*

METHODS OF OTHER INVESTIGATORS

Various methods have been reported in the literature for the growing of plants on a large-scale basis under controlled nutritional conditions. Gericke (*4*)[2] reports satisfactory results when nutrient solutions are used in shallow tanks or ponds. The plants are supported on a wire netting which is suspended at the surface of the solution. A seedbed is prepared by covering the netting with burlap and a ½-inch layer of sand. The seeds germinate in the moist sand and the roots grow through the bed into the nutrient solution. The nutrients are supplied in cartridges which slowly dissolve.

The use of an inert medium, such as sand, greatly simplifies the problem of support and aeration. In the so-called slop culture technique (*1*, *2*), the sand is put into well-drained benches or crocks and the nutrient solution poured on at intervals, the loss of water by transpiration being supplied by watering the surface of the sand. The main disadvantage of this method is that the nutrients are supplied too infrequently to insure reasonably constant osmotic concentration or balance of ions in the vicinity of the absorbing regions of the roots. Commercially, the system is wasteful of nutrients and requires more labor in watering and supplying nutrients than does soil culture.

By supplying a constant flow of solution through the sand, Shive and Stahl (*5*) were able to maintain relatively constant nutritional conditions around the roots of plants in sand culture under actively growing conditions. The method is now commonly known as the "drip culture" technique and is widely used in critical studies in plant nutrition. Crocks of sand are usually employed, the nutrient solution being supplied from a separate reservoir for each crock. The solution drips at a constant rate from a capillary tube into a modified thistle tube so that air is trapped with the solution. The method, however, does not lend itself to large-scale production because of its complexity and wastefulness of nutrients. Żurbicki (*7*) has developed an automatic method of drip culture for supplying the nutrients intermittently rather than by continuous drip. According to this method, the whole mass of sand is bathed on each cycle, and the process may be repeated as frequently as desired.

The sand-culture system described by Eaton (*3*) lends itself to larger scale production with less manipulation than the drip-culture method. It consists essentially of shallow tanks of sand fitted with perforated drainage pipes in the bottom and perforated solution

[1] Received for publication Aug. 6, 1936; issued Nov. 1, 1936.
[2] Reference is made by number (italic) to Literature Cited, p.. 701.

supply lines on the surface of the sand. The solution flows from an elevated tank through the sand into a lower tank so that the sand is thoroughly flushed at each operation. The solution in the lower tank is forced into the upper tank by reduced air pressure, maintained by a filter pump. One air pump may be used to supply a series of independent systems containing different solutions.

THE SUBIRRIGATION METHOD

The present system was developed to combine the advantages of the uniform supply of nutrients and aeration of the drip-culture technique with the relative simplicity of the slop-culture method without the attendant waste of nutrients of either system. The method has the added advantage of being capable of automatic operation over long periods of time. In principle, it consists in supplying the nutrient solution through a perforated pipe laid in the bottom of a watertight shallow bed, such as a greenhouse bench, which is filled with coarse sand, fine gravel, or cinders. The pipe is connected to a centrifugal power-driven pump which operates at intervals determined by a time clock, for a period sufficiently long barely to flood the medium, after which the clock opens the pump-motor circuit. The solution flows back through the pump by gravity into the reservoir. By the proper choice of media, the cycle may be repeated as frequently as desired, so that the roots are continuously flushed with air and nutrient solution. The essential features of the system are shown diagrammatically in figure 1.

The watertight beds may be constructed very economically by the application of asphaltic roofing materials to existing greenhouse ground beds and raised benches. Roofing asphalt is largely derived from petroleum distillation and appears to be relatively free from water-soluble toxic substances. Roofing tars, on the other hand, are mainly derived from coal distillation and contain tar acids which, together with other soluble substances, may cause severe plant injury.

All roofing asphalts are not entirely of petroleum derivation and may be modified with tars. It is wise, therefore, to find out from the manufacturer the composition of the asphaltic materials before using them in waterproofing benches. Petroleum asphalts may be obtained directly from the manufacturers of petroleum products as air-blown asphalts.

Concrete benches are easily waterproofed by painting with a cut-back asphalt (asphalt dissolved in a volatile solvent, such as naphtha), with asphalt roofing paint, or by mopping with hot fluid asphalt. The successful application of hot asphalt depends upon its being heated to a high enough temperature. Three hundred and seventy-five to four hundred degrees Fahrenheit is generally considered the correct temperature range for laying built-up roofs and is not far from the flash point, so that considerable care must be exercised.

In the construction of new benches, the concrete may be waterproofed by the addition of special asphalt preparations which are available for the purpose. The use of these preparations eliminates the necessity of surface treatment. Concrete benches are especially adaptable to the subirrigation system because the bottom of the benches may be sloped toward the center or the sides in such a way as to insure more complete drainage than could be obtained with a

level bottom. Such a sloping construction is more difficult in the case of a wooden bench.

For wooden benches an asphalt roofing paper may be used. It is most inexpensively obtained in the form of a plain felt. The rag felts rot out easily unless given a heavy coating of asphalt. The asbestos felts, while somewhat more expensive, are superior in strength

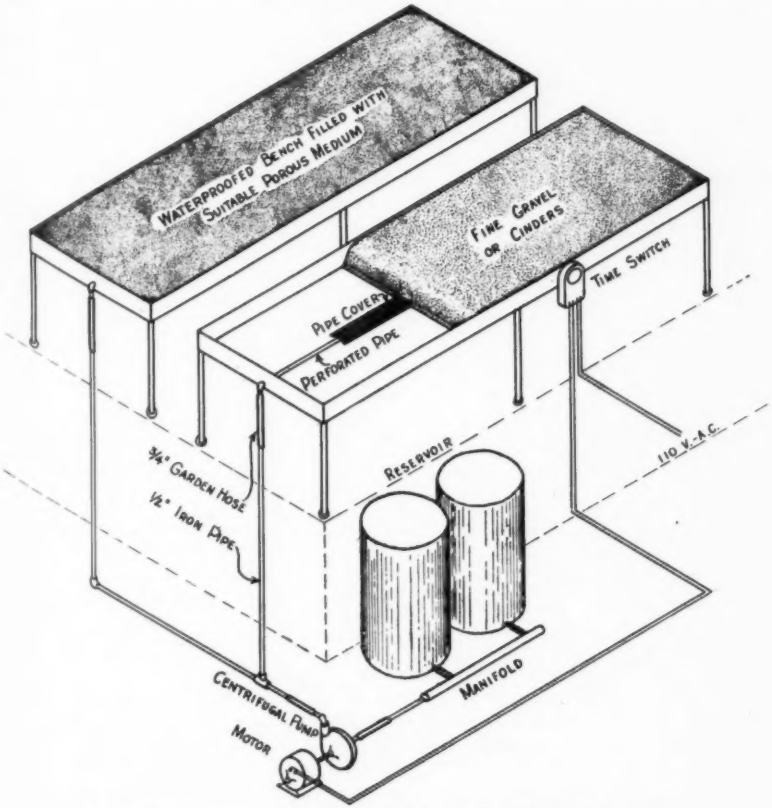


FIGURE 1.—Diagrammatic view of the subirrigation system for large-scale operation.

and decompose very slowly. In one instance, an uncoated asphalt-bound rag felt rotted so badly in 2 months that severe injury of the roots of the plants resulted from the production of hydrogen sulphide and possibly other substances of a toxic nature.[3]

The felt is so laid on the bottom of the bench and folded up over the sides as to form a complete lining. A single ply of 20-pound asbestos felt, hot-mopped on the upper surface with asphalt, is not

[3] E. F. Ries, of the Johns-Manville Corporation, recently informed the authors that tests carried on in connection with the wrapping of buried oil lines showed that, after a 15-pound asphalt-saturated rag felt had been buried for 209 days, its tensile strength decreased from 52.4 to 8.7. A similar test with an asphalt-saturated asbestos felt decreased from 39.4 to 33.2. Soil-mulching experiments with tar- and asphalt-saturated felts indicated that tar is toxic to plants while asphalt is not.

strong enough for more than a temporary set-up. For permanent waterproofing, two- or three-ply construction is necessary, hot-mopped between each layer of felt in exactly the manner of constructing built-up roofs. The most vulnerable points in the bench are the bottom edges where the felt folds up along the sides. Here the felt is weakly supported, and a 1- by 1-inch flashing strip of triangular section materially aids in preventing the tearing of the felt layers. The seams are cemented with an asphaltic asbestos roof putty. After the putty has dried, it is well to give the whole surface a coating of asphalt, using a roofing paint, or, better still, an application of hot asphalt as described for concrete benches. Coating with sand or fine gravel while the surface is still tacky is desirable.

The pipe for supplying the nutrient solution is laid on the bottom of the bench, running straight through the end boards. One end is capped so that the pipe may be flushed out in case fine sand or silt works in. The pipe is drilled with ¼-inch holes spaced 12 inches apart on the side laid next to the bottom of the bench. A 4- to 6-inch strip of heavy prepared roofing or painted felt is laid over the pipe and should run the full length. This narrow strip very effectively prevents fine material from working into the holes in the pipe line and clogging it up. A thin layer of sand or gravel will keep the strip from sticking to the bottom of the bench and will prevent interference with the flow of solution.

Black-iron pipe is useful for experimental work, since it has no toxic effect on the plants. However, it has a tendency to rust under the well-aerated conditions present. Galvanized-iron or copper pipe might be satisfactorily used. Good growth resulted in a preliminary test with copper tubing when plants were grown on a small scale, but a large-scale test has not been tried by the authors. The copper inhibited the growth of algae, which fact may in itself be an advantage. No tests have been made with galvanized-iron pipe. One pipe line is sufficient for a bench 4 to 5 feet wide filled with a fairly coarse medium. A ½-inch pipe has proved adequate for a 50-foot length of bench, with the intake at one end only. For longer benches, it would probably be necessary to use larger pipe with holes spaced closer together at the far end from the intake.

A motor-driven centrifugal pump is used to force the solution up into the beds. When the motor circuit is opened, the solution drains back through the pump into a reservoir. A ¼-horsepower pump will handle 2,500 square feet of bench filled with 5 to 6 inches of fine gravel, and less than half an hour will be required to flood the benches when the bottom of the reservoir is 4 feet below the bench. Fifty-gallon alcohol or thoroughly cleaned oil drums connected through a manifold make very useful reservoirs for the amount of solution necessary for small areas. With fine gravel or properly sifted cinders, it may generally be considered that the reservoir capacity is at least one-fourth the volume of the medium in the bench. On this basis, 1,000 square feet of bench space containing 5 inches of medium would require approximately 110 cubic feet or 800 gallons as the minimum reservoir capacity. The amount necessary varies a great deal depending upon range of particle size and proportion of coarse to fine material.

In an experimental installation, very convenient flexible connections may be made to the tanks, pump, and benches with short lengths of ¾-inch rubber garden hose slipped onto ½-inch iron pipe. Such a method of coupling does not require accurate pipe fitting and is quite easy to install.

An electric time switch set for three half-hour intervals per day is being used by the authors to control the operation of the pump. Such a system involving 1,000 square feet of bench space has operated automatically without any attention whatsoever for a week or more and the plants have remained in good condition throughout the period, being supplied with nutrients at regular intervals. The interval of feeding is largely dependent upon the nature of the experiment, the medium in the bench, the size of the plants, and the season of the year.

The most critical feature of the system is the minimum-particle size of the medium supporting the roots. Any but the finest sand will allow water to flow freely through it. However, when the roots stand in a shallow, drained bench, there may be few or no air spaces left after drainage, owing to the presence of water held by capillarity. During periods unfavorable for transpiration, as in winter, a longer time is required for the water to be transpired, and root injury due to lack of oxygen may result if the pumping cycle is repeated daily or more often. Repeating the cycle less frequently results in nonuniform nutritional conditions.

It is, therefore, essential for the satisfactory operation of a subirrigation method of nutrient supply that the supporting medium be sufficiently coarse to allow the solution to drain out fairly completely and be replaced with air after each cycle of operation. In general, it has been found advisable to remove by washing the great bulk of particles which will pass through a 16- to 20-mesh sieve (1.6 to 1.2 mm). This practically eliminates sand as a satisfactory medium, except under conditions favoring high transpiration. Fine gravel having particle sizes ranging from 1/16- to ¼-inch in diameter appears to be the most suitable for this type of culture.

The relatively inert neutral or acid gravels can be used with any of the common nutrient solutions without having much effect chemically, except possibly to supply traces of minor elements. Cinders, which have been thoroughly leached and sifted, fall in the same class. Since cinders are very porous, the upper limit of particle size may satisfactorily be in the vicinity of one-half inch for woody plants, such as rose or gardenia.

If the cinders are allowed to weather for a winter and then sifted, they may be used directly in the bench with no more than one or two thorough washings. New cinders have been used without any noticeably toxic effects by allowing water to stand for a day at a time on the benched medium. Three or four days of such washing, using clean water each time, will remove most of the soluble substances.

Calcareous gravels containing appreciable quantities of calcium carbonate have a decided influence on the nutrient solution and frequently it may be necessary to modify the solution considerably. The authors have used a fine calcareous gravel containing about 20 percent of acid-soluble material, largely calcium carbonate. In the absence of sufficient phosphate, the gravel buffers the nutrient solution in the region

of pH 8. When, however, the gravel is washed with a dilute solution of soluble phosphate to which is then added the nutrient solution, the buffering action takes place at pH 6.6 to 6.8. Possibly the surfaces of the calcium carbonate particles become coated with a mixture of calcium phosphates, which results in a buffering action at a lower pH value.

A pH value of 6.6 is quite satisfactory for many plants, but not for such plants as gardenia and some roses, which tend to become chlorotic. Attempts to increase the acidity with a soluble acid phosphate or sulphuric acid were relatively ineffective since the acid kept bringing more calcium into solution. If limestone is present in the system at all, it does not appear possible to maintain pH values much below 6.6 until it is all dissolved. This is a very important consideration when cinders or neutral or acid sands are used at low pH values. In one instance, the presence of a small amount of calcareous furnace lining in a load of cinders caused considerable difficulty by constantly raising the pH value until all of it had been dissolved with sulphuric acid.

In some sections, the readily available gravel and sand contain appreciable quantities of calcium carbonate. Such is the case in the locality in which the present investigation was made. Cinders were tried as a substitute, and thus far, they have proved, quite satisfactory for the larger woody plants. The particles are rather sharp and coarse, however, for soft herbaceous seedlings. In experimental set-ups where it is desired to obtain root weights, cinders should not be used because the particles are sharp and it is almost impossible to wash them out from the fine roots.

The element of cost becomes an important item in the use of pure chemicals when it is desired merely to get good growth. The usual three- and four-salt nutrient solutions may be readily modified to use commercial fertilizer chemicals which are inexpensive and which usually contain sufficient traces of the minor elements. The monopotassium phosphate in the Shive solutions (*6*) may be replaced with equimolarities of monocalcium phosphate as triple superphosphate and potassium sulphate. Calcium nitrate, potassium nitrate, ammonium sulphate, ammonium nitrate, ammonium phosphate, and calcium phosphate (superphosphate) are all available as fertilizer salts. Sufficient magnesium sulphate may be supplied through the use of the double salt potassium magnesium sulphate. This salt is available as a commercial fertilizer and is very useful as a source of potassium and magnesium. With these crude salts and their approximate analyses, it is possible to practically duplicate any of the usual nutrient solutions made up on a three- or four-salt basis as far as securing good growth is concerned.

The foregoing discussion has dealt mainly with large-scale production in which whole benches are supplied with the same nutrient solution by means of a power-driven pump. Small-scale experiments requiring the use of several different solutions may be set up with 18-liter bottles for reservoirs and gravity as a means of forcing the solution into the media and draining it. The bottles are made self-draining by wiring an inverted U-shaped piece of ⅛-inch black-iron pipe to the side of the bottle. A ⅜-inch rubber hose is attached to the outside end of the pipe and connected with greenhouse flats. The flats are lined with roofing paper as previously described and a perforated piece

of ¼-inch iron pipe is placed on the bottom, over which is laid a 3-inch strip of roofing paper. The ¼-inch pipe is cut 2 inches longer than the outside dimensions of the flat so that it runs through both end boards and is capped at one end. The other end connects with a short piece of ⅛-inch pipe through a reducer. Three-eighth-inch rubber tubing fits snugly on ⅛-inch standard iron pipe.

The circulation of nutrients is accomplished by raising the bottles, as shown in figure 2, until the medium containing the plants is barely flooded. The bottles are then lowered and the solution drains back. Repeating the process one to three times a day insures a relatively constant supply of nutrients to the roots and provides thorough aeration. If crocks or hard-glass beakers are used, a similar system

FIGURE 2.—Four pairs of flats (3½ by 15 by 22 inches) fed with 4 separate nutrient solutions in 18-liter bottles. The siphon is maintained by inverted U of ½-inch black-iron pipe. The flats are waterproofed with asphalt roofing paper. In the bottom of each flat is a perforated pipe over which is placed a 3-inch strip of heavy roofing paper to keep out fine material.

may be employed involving only glass, quartz sand, and rubber tubing. In this case, a siphon is used in the beakers of sand also.

Figure 3 shows a similar set-up in which the plants are growing in individual containers. The buckets are painted on the inside with asphalt paint and supplied with perforated tubing covered with a disk of asphalt roofing. The nutrients are supplied from a 40-liter crock suspended by a pulley arrangement. By the use of counterweights, the crock may be lowered and raised with relative ease. When several buckets or flats are connected together, one will drain first and sometime break the siphon. A second return of small tubing dipping into the top of the reservoir will satisfactorily drain the system. The advantage of growing plants in individual containers is that it enables the experimenter to remove any container desired at any time for special treatment.

SUMMARY

A subirrigation method of supplying nutrient solutions to plants growing in sand culture which lends itself to large-scale production is described. It consists in principle of pumping nutrients from a submerged reservoir into the bottom of a shallow bed of fine gravel or cinders with a centrifugal pump. The pump is controlled by an electric time switch which stops the operation when the bench is flooded. The solution then flows back into the tank through the pump by gravity.

FIGURE 3.—A series of buckets and crocks fed with nutrients from a 40-liter crock which is raised and lowered by a counterbalanced system of pulleys. The solution flows in through a ¾-inch hose connected to a ½-inch iron manifold to which is soldered ⅛-inch iron pipe for supplying the containers of fine gravel. The small tubing allows the solution to drain back in case air should break the siphon.

Modifications of the method are discussed for use in experimental set-ups on a small scale, in which the solution flows into and out of the plots by gravity through the raising and lowering of bottles of solution.

The advantages of the system are (1) frequent and complete flushing of the roots with air and nutrient solution, (2) economy of nutrients since the solution drains back into the tank again, and (3) completely automatic operation over long periods of time.[4]

[4] Since the foregoing manuscript was submitted for publication several improvements in the method have been made.

Wooden benches may be very permanently waterproofed with hot asphalt and gravel. The cracks in the bench are lightly covered with lath and the bottom leveled off with a thin layer of fine gravel. Asphalt heated to 400° F is then applied over the whole surface with a sprinkling can. At this temperature the asphalt flows readily through the fine holes of a nozzle. A second layer of gravel and asphalt is then applied. One and one-half to two pounds of asphalt is required per square foot. The dry, tamped soil of ground beds has been satisfactorily treated in the same manner. Six-inch boards are used as sides. The nutrient solution may be more uniformly supplied to the bench by substituting for the perforated iron pipe and cover a single line of inverted 4-inch double-beaded galvanized roofing gutter laid on the bottom of the bench so as to form a large open channel for the unimpeded flow of solution. A short piece of ½-inch pipe sealed in the end of the bench admits the solution. Before the gutter is installed it should be given several coats of asphalt roofing paint to keep the solution from direct contact with the zinc surface, which has been found to be toxic to plants in highly acid solutions. Such a large open channel is easier to install than pipe and shows little tendency to clog with roots.

LITERATURE CITED

(1) Biekart, H. M., and Connors, C. H.
1935. The greenhouse culture of carnations in sand. N. J. Agr. Expt. Sta. Bull. 588, 24 pp., illus.
(2) Connors, C. H., and Biekart, H. M.
1931. Roses in sand culture. N. J. Agr. Expt. Sta. Ann. Rept. (1930–31). 52: 293–296, illus.
(3) Eaton, F. M.
1931. A large sand culture apparatus. Soil Sci. 31: 235–241, illus.
(4) Gericke, W. F.
1929. Aquaculture: a means of crop-production. (Abstract) Amer. Jour. Bot. 16: 862.
(5) Shive, J. W., and Stahl, A. L.
1927. Constant rates of continuous solution renewal for plants in water cultures. Bot. Gaz. 84: 317–323, illus.
(6) Spencer, E. L., and Shive, J. W.
1933. The growth of rhododendron ponticum in sand cultures. Bull. Torrey Bot. Club 60: 423–439, illus.
(7) Żurbicki, Z.
1933. A new method of arranging experiments with vegetables in sand cultures with flowing solutions. Plant Physiol. 8: 553–558, illus.

114406—36——5

ROLE OF INSECTS IN THE DISTRIBUTION OF COTTON WILT CAUSED BY FUSARIUM VASINFECTUM [1]

By J. J. TAUBENHAUS, *chief, Division of Plant Pathology and Physiology, Texas Agricultural Experiment Station*, and L. DEAN CHRISTENSON, *field assistant, Division of Cotton Insect Investigations, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture*

INTRODUCTION

The methods of spread of cotton wilt caused by *Fusarium vasinfectum* Atk. have received considerable attention from investigators. It has long been suspected that insects frequently play a part in the local spread of cotton wilt. During 1933 and 1934 the writers sought to obtain definite information on the possibility of spread of wilt through the fecal pellets of insects that normally feed on the different parts of cotton plants known to be infected with fusarium wilt. Some attention was also given to spread of *F. vasinfectum* as adhering spores on the exterior parts of the body of cotton insects. The laboratory studies here reported were made at College Station, Tex. The field data were obtained in cotton fields near College Station, Beaumont, and Caldwell, Tex. Brief reports on this work appeared in 1933 and 1934.[2]

METHODS AND MATERIALS

The experiments were divided into three groups dealing with cotton insects feeding on (1) roots, (2) stems and leaves, and (3) bolls. The few species worked with were selected because of the ease with which they could be submitted to experimental conditions.

In the laboratory, field-collected insects were caged with portions of roots, stems, or leaves of cotton plants known to be infected with *Fusarium vasinfectum*. The fecal pellets from these insects were collected at least once a day and dried so that they would not quickly disintegrate when placed in fluids. Then they were surface-sterilized by being placed for 30 seconds in an aqueous solution containing, by weight, 0.05 percent mercuric chloride and 35 percent ethyl alcohol, and finally, after being rinsed three times in sterile water, they were planted in Petri plates on acidified potato-dextrose agar. In other tests, certain cotton insects were allowed to feed on normal cotton leaves that had been sprayed with a heavy suspension of spores of a pure culture of *F. vasinfectum*. The fecal pellets from such insects were collected each morning and cultured in the same manner. From time to time insects were dissected under aseptic conditions, and fecal pellets about to be defecated were extracted and cultured. In other tests, insects were permitted to feed on wilt-infected cotton material, and then, after prolonged surface sterilization, the entire insects were cultured on nutrient agar in Petri dishes.

[1] Received for publication July 8, 1936; issued Nov. 1, 1936. Contribution no. 291, Technical Series, Texas Agricultural Experiment Station.

[2] TAUBENHAUS, J. J., and CHRISTENSON, L. D. EFFECT OF INSECTS AND OTHER ANIMAL ORGANISMS ON THE SURVIVAL OF THE CAUSATIVE ORGANISM OF COTTON WILT, FUSARIUM VASINFECTUM. Tex. Agr. Expt. Sta. Ann. Rept. 46: 89-90. 1933.

——— and CHRISTENSON, L. D. INSECTS AS POSSIBLE DISTRIBUTING AGENTS OF COTTON WILT CAUSED BY FUSARIUM VASINFECTUM. (Abstract) Phytopathology 24: 839-840. 1934.

In field tests designed to determine whether cotton insects act as carriers of *Fusarium vasinfectum*, a number of insects were collected in wilt-infected fields. Where possible they were picked up with sterile forceps. To obtain very active species it was necessary to net them or bat them down with a swatter. Each individual thus secured was placed in a sterile vial plugged with cotton and brought to the laboratory, surface-sterilized, and then cultured, or fecal pellets about to be defecated were dissected out and cultured. Soil-inhabiting insects in wilt-infested cotton fields were collected by means of a Berlese funnel and then surface-sterilized and cultured.

Wherever possible, precautions were taken to prevent or minimize casual contamination of fecal pellets or insects to be cultured. All cages and vials were heat-sterilized before use. In experiments with white grubs the soil in which the insects were confined was heat-sterilized at intervals. Spore-covered leaves used for the leaf-feeding insects were suspended at the tip of vials to prevent falling fecal pellets from coming in contact with the leaf areas.

EFFECTIVENESS OF STERILIZATION METHOD

It was established early in these experiments that an aqueous solution containing, by weight, 0.05 percent mercuric chloride and 35 percent ethyl alcohol was effective in destroying the hyphae, chlamydospores, and the microspores as well as the macrospores of *Fusarium vasinfectum*. This solution, however, did not always spread uniformly over the entire surface of fecal pellets or of entire insects when these were characterized by deep sutures or by hirsute bodies or appendages.

Tests to determine the effectiveness of surface sterilization were made by covering entire insects and fecal pellets with a spore suspension of *Fusarium vasinfectum*. One lot each of larvae and fecal pellets of *Alabama argillacea* Hbn. thus treated was surface-sterilized with the mercuric chloride-alcohol solution with 100-percent effectiveness, while in a lot of grasshoppers similarly treated sterilization was only 74 percent effective. Results of the many experiments subsequently made, however, indicated that in the main this sterilization method was highly effective. Nevertheless, external sterilization alone was not relied on. The results were checked from time to time by culturing fecal pellets dissected out from the recta of insects under aseptic conditions.

IDENTITY AND PATHOGENICITY OF THE FUSARIA

All isolations of the fusaria in these experiments were finally grown in tubes on potato-dextrose agar or on sterilized rice. Morphologically, and in pure culture, the strains of *Fusarium* recovered from the fecal pellets or insects used in these tests could not be distinguished from typical *F. vasinfectum* isolated from the roots or stems of a naturally infected cotton plant. Many of these strains were identified by Dr. C. D. Sherbakoff, of the Tennessee Agricultural Experiment Station, as *F. vasinfectum*, but somewhat different from *F. vasinfectum* that affects cotton in Tennessee. Cotton seedlings were successfully inoculated with pure cultures of *F. vasinfectum* obtained from Sherbakoff. Successful inoculations were also made with strains of *Fusarium* recovered by the writers at College Station and Beaumont from insects that had fed on normally infected cotton

plants and from the fecal pellets of these insects. It is of interest to note that more than 80 percent of the fusaria recovered from the fecal pellets of the cotton insects used in these experiments were the same as or similar to *F. vasinfectum*. Other organisms recovered, particularly from the fecal pellets, were *F. semitectum* and various species of *Aspergillus*.

In November 1934 several thousand fecal pellets were collected from grasshoppers that had fed on cotton stems naturally infected with cotton wilt. These pellets were kept at room temperature, in a test tube plugged with cotton, and cultures were made of them from time to time. The percentage of pellets from which *F. vasinfectum* was recovered is shown in table 1. It will be noted that *F. vasinfectum* remained viable for at least 15 months.

TABLE 1.—*Viability of Fusarium vasinfectum in dried fecal pellets obtained November 1934 from grasshoppers fed on wilt-infected cotton stems*

Date cultured	Pellets cultured	Pellets from which *F. vasinfectum* was obtained	
	Number	*Number*	*Percent*
December 1934	160	147	91.9
April 1935	210	180	85.7
November 1935	117	93	79.5
March 1936	287	117	40.8

RECOVERY OF FUSARIUM VASINFECTUM FROM ROOT-FEEDING INSECTS

The root-feeding insects used included white grubs (*Phyllophaga crassissima* Blanch. and other unidentified species), wireworms, and numerous small insects usually included in the category of "smaller soil animals."

The white grubs were placed in soil cages and fed on portions of cotton roots infected with *Fusarium vasinfectum*. The fecal pellets were collected daily by soil sifting and cultured in the usual way. As shown in table 2, out of 239 fecal pellets cultured, 78, or 32.6 percent, yielded pure cultures of *F. vasinfectum*. Likewise, cultures were made of 17 entire white grubs that had been feeding on infected cotton roots, and 4, or 23.5 percent, yielded pure growth of *F. vasinfectum*.

TABLE 2.—*Petri-dish cultures of fecal pellets of white grubs (Phyllophaga crassissima and others) fed on portions of cotton roots infected with fusarium wilt*

Degree and type of feeding	Fecal pellets cultured	Cultures showing good growth of *Fusarium vasinfectum*	
	Number	*Number*	*Percent*
Slight to moderate surface grazing	17	1	5.9
	1	1	100.0
	16	16	100.0
	3	2	66.7
Moderate gouging	3	0	0.
	3	0	0.
	11	5	45.5
	12	0	0.
	12	8	66.7
Copious gouging	119	20	16.8
	17	17	100.0
	25	8	32.0
Total	239	78	32.6

A number of wireworms were placed in soil cages and permitted to feed on the roots of wilt-infected cotton plants. After feeding, they were surface-sterilized and the entire insects cultured. In not a single instance was *Fusarium vasinfectum* recovered.

Among the smaller soil animals worked with were the Collembola (including *Onychiurus fimetarius* (L.) Lubb., *Pseudosinella violenta* Fols., and *Entomobrya sabulicola* Mills [3]), a species of Japygidae, and coleopterous larvae. They were collected from the soil about the roots of wilted cotton plants and cultured with and without surface sterilization. Certain of the Collembola, having shown a marked avidity for mycelial growth of *Fusarium vasinfectum*, were fed for from 3 to 24 days on pure slant cultures of the cotton-wilt fungus. General collections of smaller insects obtained from the soil surrounding wilt-infected cotton roots were surface-sterilized and also cultured. The results are shown in table 3. It will be noted that, of all the specimens of Collembola cultured, only one (*P. violenta*), which was not surface-sterilized, yielded growth of *F. vasinfectum*. Growth of *F. vasinfectum* was obtained from two of four unidentified coleopterous larvae cultured. In cultures of approximately 1,000 smaller soil insects and other Arthropoda, 6 yielded growth of *F. vasinfectum*.

TABLE 3.—*Petri-dish cultures of smaller soil animals collected from soil under wilted cotton plants or fed upon pure cultures of Fusarium vasinfectum prior to culturing*

Species	Source or treatment of insects	Preparation for culturing	Insects cultured	Insects yielding growth of *Fusarium vasinfectum*	
			Number	*Number*	*Percent*
Onychiurus fimetarius	Soil under wilted plant	Not sterilized	(1)	0	
Pseudosinella violenta	do	do	7	1	14.3
	do	Washed and sterilized	17	0	
	Fed on *F. vasinfectum*	Washed in sterile water	4	0	
	do	Washed and sterilized	15	0	
Entomobrya sabulicola	Soil under wilted plant	do	2	0	
	Fed on *F. vasinfectum*	Not sterilized	7	0	
	do	Washed in sterile water	4	0	
	do	Washed and sterilized	18	0	
Japygidae	Soil under wilted plant	Not sterilized	5	0	
Coleopterous larvae	do	Washed and sterilized	4	2	50.0
Miscellaneous smaller soil animals, etc.[2]	do	do	[3] 1,000	6	.6

[1] Several.
[2] Composed of Collembola, Japygidae, Campodeidae, larval stages, etc. Other arthropods, such as Pauropoda, Symphyla, Diplopoda, Chilopoda, Acarina, Araneida, etc., were included.
[3] Approximate number.

RECOVERY OF FUSARIUM VASINFECTUM FROM STEM- AND LEAF-FEEDING INSECTS

The stem borer *Ataxia crypta* Say [4] is frequently found infesting dead stems of cotton plants in Texas and consuming tissues normally attacked by the cotton-wilt fungus. In feeding within cotton stems this insect usually expels considerable frass through a small hole maintained for the purpose. The frass falls to the ground or is blown away by the wind. A number of *A. crypta* were introduced into stems of cotton plants that had been killed by *Fusarium vasinfectum*. Nine entire larvae, fecal pellets, and frass were surface-

[3] Identified by J. W. Folsom, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture.
[4] Identified by H. J. Reinhard, Texas Agricultural Experiment Station.

sterilized and cultured in the usual way. *F. vasinfectum* was recovered from the frass and from six of the nine entire larvae.

Among the grasshoppers [5] commonly found in wilt-infected cotton fields in Texas are the following: *Melanoplus differentialis* Thos., *M. mexicanus* Sauss., *M. femur-rubrum* Deg., *Encoptolophus texensis* Brun., *Spharagemon cristatum* Scudd., *Tomonotus aztecus* Sauss., *Chortophaga viridifasciata* var. *australior* Deg., *Schistocerca americana* Dru., *S. obscura* Fab., *Trimerotropis citrina* Scudd., and *Dissosteira carolina* L.

A number of grasshoppers of the species *Melanoplus femur-rubrum*, *M. differentialis*, and *Schistocerca obscura* and an unidentified miscellaneous group were caged and permitted to feed on the stems of wilt-infected cotton plants. The insects were forced to consume diseased tissues or starve, inasmuch as all foliage had been removed. The fecal pellets were collected each morning and cultured immediately after surface sterilization. As shown in table 4, 25 lots containing a total of 1,729 fecal pellets, were cultured. Pure growth of *F. vasinfectum* was recovered from 80 percent of these lots. In addition, four surface-sterilized adult grasshoppers were cultured, one of which produced a growth of *F. vasinfectum* originating at a point on a tarsus.

TABLE 4.—*Petri-dish cultures of fecal pellets of grasshoppers fed on portions of cotton stems infected with fusarium wilt*

Species	Fecal pellets cultured	Growth of *Fusarium vasinfectum* [1]
	Number	
Melanoplus differentialis	36	−
	59	+
	56	+
	42	−
	85	+
	50	−
	39	+
	51	+
	40	+
	49	+
	100	+
	100	+
	72	+
	149	+
	164	+
Melanoplus femur-rubrum	50	+
	205	+
Schistocerca obscura	18	+
	12	+
Miscellaneous [2]	44	+
	57	−
	46	−
	55	+
	100	+
	50	+
Total	1,729	5 −, 20 +

[1] + denotes positive growth of *F. vasinfectum*, − no growth.
[2] Chiefly *Melanoplus differentialis;* also *M. femur-rubrum* and unidentified nymphs.

During the season of 1934 field studies were made to determine the extent to which leaf-feeding insects in badly diseased cotton areas may act as carriers of cotton wilt. Grasshoppers, including all the

[5] Identified by the late A. N. Caudell, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture.

species mentioned above, were used in these experiments, because they are active fliers, are prevalent in wilt-infected cotton fields in Texas, and are also known to consume quantities of cotton-leaf tissue. Pure cultures of *Fusarium vasinfectum* were obtained from 22.2 percent of 419 grasshoppers collected near Caldwell, from 26.6 percent of 184 collected near College Station, and from 33.3 percent of 90 obtained near Beaumont. In the Caldwell field, from which data of a seasonal nature were obtained, 9.4 percent of the grasshoppers were found to carry the wilt fungus internally on July 24, 11.7 percent on August 7, 35.6 percent on September 4, 24.9 percent on September 27, and 29.5 percent on October 18. These statements apply only to those species of grasshopper for which positive results were obtained. Several other species that were present in the fields in smaller numbers were not tested.

There was no way of determining whether the number of grasshoppers acting as carriers of *Fusarium vasinfectum* was in direct ratio to the number of plants infected in the field. All fields in which surveys were made proved ultimately to have 75 percent or more of the plants diseased.

Larvae of *Alabama argillacea*, *Laphygma frugiperda* S. and A., and *Prodenia ornithogalli* Guen. obtained from nonwilt areas were placed in separate cages and fed on cotton leaves naturally infected with wilt. In addition, larvae of *Alabama* and *Prodenia* were fed on leaves that had been painted with a heavy suspension of spores of a pure culture of *Fusarium vasinfectum*. The fecal pellets and some of the entire insects were cultured in the usual way. As shown in table 5 and figure 1, typical *F. vasinfectum* was often recovered from the fecal pellets of the larvae and from some of the entire insects.

TABLE 5.—*Petri-dish cultures of fecal pellets and various stages of lepidopterous leaf-feeding insects*

Species	Kind of food consumed	Material	Cultures	Cultures showing good growth of *F. vasinfectum*	
			Number	*Number*	*Percent*
Laphygma frugiperda	Naturally infected cotton leaves.	Fecal pellets	5	0	0
			29	16	55.2
			40	0	0
			114	43	37.7
			8	8	100.0
Prodenia ornithogalli	do	do	1,826	559	30.6
	do	Entire larvae	20	1	5.0
	Spore-covered cotton leaves.	Fecal pellets	52	29	55.8
Alabama argillacea	Naturally infected cotton leaves.	do	768	615	80.0
	do	Entire larvae	9	2	22.2
	Spore-covered cotton leaves.	Fecal pellets	202	202	100.0
	do	Entire larvae	2	0	0
	do	Prepupae	1	0	0
	do	Pupae	10	1	10.0
	do	Adults	4	0	0

Tests were made to determine whether the wilt organism could remain within *Alabama argillacea* during pupation and later be disseminated by the adult moth, which has exceptional migratory powers. This problem was attacked by determining the degree of completeness with which the alimentary tract was voided of viable fungus material through the elimination processes. Larvae were fed

for a time upon normal cotton leaves, then upon spore-painted leaves, and then once more upon normal leaves. Fecal pellets were cultured individually as defecated. No growth of *Fusarium vasinfectum* was obtained until approximately 26 minutes after spore-covered cotton leaves had been provided as food. All fecal pellets were then found to sponsor growth of the fungus until approximately 26 minutes from the time the second feeding on normal leaves had begun. Thereafter no growth of *F. vasinfectum* was obtained. In other tests larvae were allowed to feed on spore-covered cotton leaves and then starved for

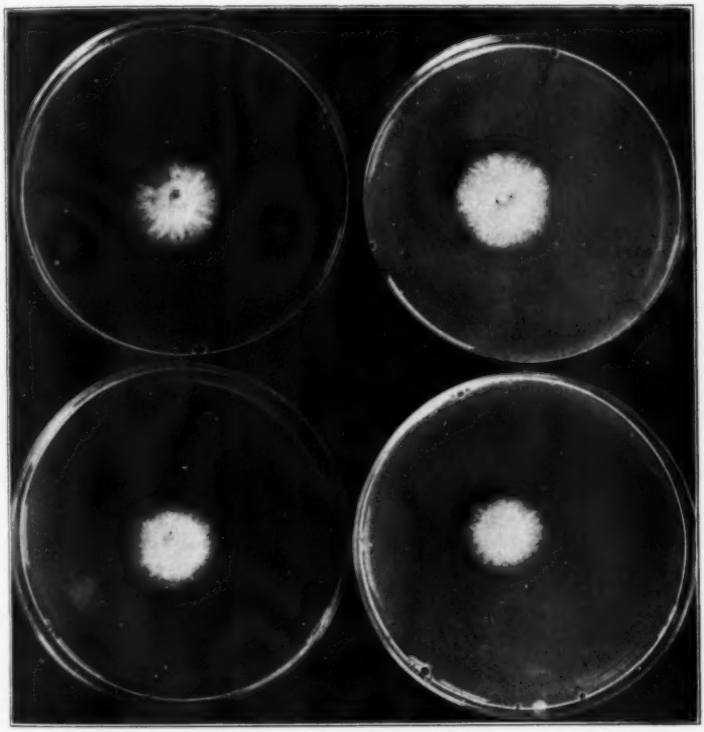


FIGURE 1.—Pure cultures of *Fusarium vasinfectum* recovered from individual fecal pellets of *Alabama argillacea* larvae that had been fed on cotton leaves painted with a suspension of the spores of *F. vasinfectum*.

from 1 to 4 days. These larvae were then dissected and their alimentary tracts aseptically removed and cultured. The results were always negative.

Similar tests were made with *Melanoplus differentialis* and *Schistocerca americana*, with the same results. In the case of *M. differentialis* no growth of *Fusarium vasinfectum* was obtained in cultures after approximately 45 minutes from the time the last spore-covered leaves had been eaten. For *S. americana* the period was 42 minutes (at 26.5° C.).

From these results it appears that the passage of *Fusarium vasinfectum* through the alimentary tracts of the insects used in these tests was relatively rapid.

This conclusion is substantiated by the results of cultures of pupae and adults of *Alabama argillacea*, the larvae of which had been fed upon spore-contaminated leaves for several days prior to pupation. In cultures of 49 of these pupae that had spun up in heavily inoculated leaves, only 2 yielded growth of *F. vasinfectum*. In cultures of 20 adults that were collected as they emerged from their pupal cases, no wilt fungus was obtained. There is little likelihood that the fungus is retained within *A. argillacea* in a viable state during pupation and formation of the imago.

RECOVERY OF FUSARIUM VASINFECTUM FROM BOLL-FEEDING INSECTS

Boll-feeding insects are usually numerous in cotton fields. Two types were worked with, the boll weevil (*Anthonomus grandis* Boh.) and the bollworm (*Heliothis obsoleta* Fab.). Only entire immature stages and adult boll weevils collected from within bolls on badly wilted cotton plants were cultured. Three out of ninety-five larvae, or 3.2 percent, 1 out of 30 pupae, or 3.3 percent, and 1 out of 61 adults, or 1.6 percent, yielded pure-culture growth of *Fusarium vasinfectum*. The results of these tests with the boll weevil are not considered as conclusive of the passage of viable fungus through the alimentary tract and need further verification. Fecal pellets from *H. obsoleta* larvae that had fed upon spore-covered involucral bracts and small bolls were cultured after external sterilization. Pure growth of *F. vasinfectum* was obtained from 60 percent of these.

DISSEMINATION OF FUSARIUM VASINFECTUM THROUGH EXTERNALLY ADHERING SPORES

Numerous tests were made to determine the ability of insects to spread the cotton-wilt fungus on their appendages. After being exposed to cotton stems bearing an abundance of sporodochia, such insects as *Anthonomus grandis* Boh., *Olla abdominalis* Say, *Jadera haematoloma* H. S., *Zelus cervicalis* Stål, *Nezara viridula* L., *Euschistus servus* Say, and others were found to start cultures of *Fusarium vasinfectum* where their feet were permitted to touch the surface of acidified agar in Petri dishes. Cultures of the appendages of insects captured in badly wilted cotton fields showed that many normally transport viable fungus in this manner.

DISCUSSION

This paper has indicated the possibility of insects spreading *Fusarium vasinfectum* through their fecal pellets. Such pellets may carry the fungus as bits of hyphae or spores, which are unaffected by brief surface sterilization with an aqueous solution containing, by weight, 0.05 percent mercuric chloride and 35 percent ethyl alcohol.

To check the reliability of the results, cultures were made of fecal pellets dissected from the recta under aseptic conditions, and typical growth of *Fusarium vasinfectum* was obtained from the following insects: *Schistocerca americana*, *Melanoplus differentialis*, *M. mexi-*

canus, *Chortophaga viridifasciata* var. *australior*, *Encoptolophus terensis*, *Trimerotropis citrina*, and *Spharagemon cristatum*.

That the results obtained are largely accurate is further indicated by the negative results obtained with certain soil-inhabiting insects. If the methods had allowed contaminations sufficient to account for the large percentage of positive results obtained in many experiments, one might expect that those tests which were habitually negative would also have been subject to the same degree of contamination and that positive results would have been intermixed with negative ones. The same logic applies to the test in which cultures were made of individual pellets, as these were defecated from larvae of *Alabama argillacea* and grasshoppers that had been fed successively upon wilt-free food, spore-covered food, and wilt-free food. If the positive results had originated in external contaminations, there would have been no such abrupt change in the positive or negative character of cultures.

Another test may be cited as an indication of the accuracy of the results. Living *Alabama argillacea* larvae, containing wilt fungus internally, were submerged in the sterilization fluid for 30 seconds. This exposure was found to be not immediately fatal. The larvae were then transferred with sterile forceps to sterile acid-agar plates, where they were left until they had defecated one or more fecal pellets. The insects were then removed and the plate was left for fungus development. All of a number of tests resulted positively. In every instance the growth of *Fusarium vasinfectum* originated with fecal pellets. That external sterilization was efficient is indicated by the fact that not a single growth of the wilt fungus resulted from contact of larvae with agar surfaces during their wanderings inside the Petri dishes.

The point of appearance of *Fusarium vasinfectum* in cultures also merits consideration. When entire insects were externally sterilized and cultured, growths of the wilt fungus most commonly made their appearance near the anus, and more rarely on appendages and external body parts.

Passage of the cotton-wilt fungus through the alimentary tract of the insects studied appears to have been relatively rapid. Swiftly flying insects could undoubtedly spread *Fusarium vasinfectum* from field to field through their fecal pellets, although the supply of fungus would be exhausted before they could cover great distances. Those insects that do carry wilt fungus internally are probably of importance in field-to-field spread, and in intensifying the infective element within a field. Each infective fecal pellet deposited is an additional source of fungus that may ultimately attack cotton plants. The fecal pellet itself provides sufficient nutrient material to enable the fungus to grow. The possibilities with respect to insects that serve as vehicles for externally adhering spores are almost unlimited.

It is worthy of note that those insects whose alimentary fluids had no immediate lethal effect on *Fusarium vasinfectum* are all phytophagous. Most of the insects that destroyed wilt fungus by eating it, particularly the Collembola, feed normally upon decaying organic matter and fungi. Indications are that the association between insects and the wilt fungus is entirely a mechanical one.

SUMMARY

The following cotton insects were caged and fed on roots, stems and leaves, or bolls of cotton plants infected with typical fusarium wilt: *Melanoplus femur-rubrum, M. differentialis, Schistocerca americana, S. obscura,* and other less abundant grasshoppers; the larval stages of *Alabama argillacea, Laphygma frugiperda,* and *Prodenia ornithogalli;* a number of species of white grubs; and the larval stage of *Ataxia crypta.* Viable *F. vasinfectum* was recovered from fecal pellets or entire insects cultured on potato-dextrose agar in Petri dishes. The wilt fungus could not be recovered from, and was apparently destroyed while passing through, the alimentary tract of wireworms, Collembola, and Japygidae.

Viable *Fusarium vasinfectum* was recovered from cultures of the entire insects or of fecal pellets dissected out from the recta of numerous species collected in badly wilted cotton fields in Texas. The following insects were found to act as natural carriers of the cotton wilt fungus: *Melanoplus differentialis, M. mexicanus, M. femur-rubrum, Encoptolophus texensis, Spharagemon cristatum, Tomonotus aztecus, Chortophaga viridifasciata* var. *australior, Schistocerca americana, S. obscura, Trimerotropis citrina,* and *Dissosteira carolina.* It still remains to be proved whether the boll weevil can act as a carrier of fusarium wilt of cotton.

Cotton seedlings were successfully inoculated with strains of *Fusarium* isolated from the alimentary tract of insects and with a pure culture of *F. vasinfectum* from infected cotton.

Fusarium vasinfectum has survived for 15 months in fecal pellets from grasshoppers fed on wilt-infected cotton stems and kept dry in the laboratory.

It is suggested that these results may help to explain the occasional finding of infected plants in areas where the disease does not ordinarily occur.

RELATION OF THE CAROTENE CONTENT OF CERTAIN FEED MATERIALS TO THEIR VITAMIN A POTENCY [1]

By G. S. Fraps, *chief, Division of Chemistry,* Ray Treichler, *assistant chemist,* and A. R. Kemmerer, *assistant chemist, Texas Agricultural Experiment Station*

INTRODUCTION

It is now known that alpha-, beta-, and gamma-carotene, cryptoxanthin, and some other yellow pigments of plants can be utilized for the same purposes as vitamin A (*5*, *7*).[2]

Carotene can be determined in a few hours, whereas the biological determination of vitamin A potency requires several weeks. Variations in carotene content can be easily ascertained chemically. The determination of carotene therefore offers a rapid method for estimating the vitamin A potency of many feeds, provided the relation between the carotene content of a particular feed and its biological value is known. Hence the relation between the carotene content of feeds and their vitamin A potency is of considerable practical as well as scientific importance. In connection with vitamin A investigations at the Texas Agricultural Experiment Station, it was necessary to determine the vitamin A potency of a number of feeds. Advantage was taken of the material available to determine the carotene content also and to secure information regarding the ratio mentioned.

EXPERIMENTAL PROCEDURE

The feeds were assayed biologically for vitamin A potency by means of the modified Sherman-Munsell method employed in this laboratory (*2*). The results were expressed both as Sherman-Munsell units and as international units. The international units were calculated by multiplying the number of Sherman-Munsell units by 1.2. In this laboratory, 1.2 international units have been found to be equal to 1 Sherman-Munsell unit.

Some of the feeds tested were ground and stored at room temperature during the fall, winter, and spring months; others were stored in an electric refrigerator. The carotene was determined at approximately the same time that the biological assays were made in order to minimize any error that might result from loss of carotene during storage.

The carotene in the various samples was determined by a modification of the method worked out by Guilbert (*3*) consisting in brief as follows: 2 to 5 grams of the feed sample was refluxed with aldehyde-free alcoholic potash for 30 minutes. The carotinoids were then extracted from the resulting solution by diluting with water and shaking with ethyl ether. The ether was then washed with distilled water and evaporated off under reduced pressure. The residue remaining was taken up in light petroleum ether and the xanthophyll removed by shaking with 85 and 90 percent methanol. The carotene

[1] Received for publication June 15, 1936; issued Nov. 1, 1936. Technical contribution no. 354 of the Texas Agricultural Experiment Station.

[2] Reference is made by number (italic) to Literature Cited, p. 716.

was determined by comparing the petroleum ether solution in a colorimeter with a 0.1 percent solution of potassium dichromate which had been standardized against 0.001 percent carotene solution. All the results obtained were expressed as micrograms of carotene per gram of feed. No attempt was made to differentiate between the several active pigments.

RESULTS

A total of 15 samples of feeds are reported for carotene and vitamin A potency. There were seven samples of alfalfa meal, five samples of peanut hay, two samples of yellow corn, and one sample of Johnson grass hay.

The carotene of the alfalfa varied from 7.3 to 63.5 micrograms per gram, while the vitamin A potency varied from 13 to 77 Sherman-Munsell units (15.6 to 92.4 international units) per gram. The data are given in table 1. In the seven samples of alfalfa products, 1 microgram of carotene had a vitamin A potency of from 1.0 to 1.8 Sherman-Munsell units (1.2 to 2.1 international units) with an average of 1.4 Sherman-Munsell units (1.6 international units). The international units per microgram of carotene were secured by dividing the international units per gram of feed by the microgram of carotene per gram of feed. If the Sherman-Munsell units per microgram of carotene are multiplied by 1.2, slightly different results are secured in some cases because of the rounding off of the fractions before the Sherman-Munsell units per microgram of carotene were tabulated.

TABLE 1.—*Relation of carotene in feeds to their vitamin A potency*

Laboratory no.	Kind of feed	Vitamin A potency per gram feed		Micrograms of carotene per gram of feed	Units of vitamin A potency per microgram of carotene	
		Sherman-Munsell units	International units		Sherman-Munsell units	International units
41727	Alfalfa meal	60	72.0	48.1	1.2	1.5
41745	Alfalfa leaf meal	67	80.4	53.1	1.3	1.5
41952	Alfalfa hay	13	15.6	7.3	1.8	2.1
41967	Dehydrated alfalfa leaf meal	77	92.4	59.4	1.3	1.6
42622	do	75	90.0	63.5	1.2	1.4
41728	Alfalfa leaf meal	25	30.0	25.0	1.0	1.2
41277	Dehydrated alfalfa leaf meal	60	72.0	33.6	1.8	2.1
	Mean				1.4	1.6
41724	Peanut hay	8	9.6	5.6	1.4	1.7
41726	do	17	20.4	13.3	1.3	1.6
41732	do	38	45.6	26.5	1.4	1.7
41733	do	15	18.0	13.9	1.1	1.3
41734	do	25	30.0	21.4	1.2	1.4
	Mean				1.3	1.5
41110	Yellow corn	2	2.4	1.7	1.2	1.4
41229	do	3	3.6	2.6	1.2	1.4
	Mean				1.2	1.4
41951	Johnson grass hay	10	12.0	9.3	1.1	1.3

Peanut hay ranged in vitamin A potency from 5.6 to 26.5 micrograms of carotene per gram and from 1.1 to 1.4 Sherman-Munsell units (1.3 to 1.7 international units) per microgram of carotene with an average of 1.3 (1.5 international units) for the five samples.

The two samples of yellow corn had a value of 1.2 Sherman-Munsell units (1.4 international units) of vitamin A potency per microgram of carotene, while the sample of Johnson grass hay had a vitamin A potency of 1.1 Sherman-Munsell units (1.3 international units) per microgram of carotene.

Results from feeding rats purified carotene dissolved in oil show that 1.0 Sherman-Munsell unit is equivalent to 0.72 microgram of beta-carotene. This makes 1 microgram of beta-carotene equal to 1.4 Sherman-Munsell units (table 2). The same results were obtained by feeding the international standard. One Sherman-Munsell unit was found to equal 1.2 international units. Since 1 international unit is 0.6 microgram of beta-carotene, 1.2 international units are equal to 0.6×1.2 or 0.72 microgram of beta-carotene. Since the average value of 1 microgram of carotene in alfalfa products and in peanut hay was 1.4 and 1.3 Sherman-Munsell units, respectively, the biological value of carotene in the international standard, in the purified carotene, and in the alfalfa and peanut hay products are practically the same. The differences found are within the limits of experimental error. This result is similar to that of Morgan and Madsen (*8*), who found the value of the carotene in apricots to be nearly 100 percent of the expected value.

TABLE 2.—*Relation of carotene content to vitamin A potency when rats were fed various feeds and purified carotene in oil*

Material	Vitamin A potency per microgram of carotene	
	Sherman-Munsell units	International units
Alfalfa products	1.4	1.6
Peanut hay	1.3	1.5
Yellow corn	1.2	1.4
Johnson grass hay	1.1	1.3
International standard	1.4	1.7
Purified carotene in oil	1.4	1.7

Practically all the carotene of alfalfa hay and peanut hay should be beta-carotene, as has already been reported for alfalfa hay by Hartman, Kane, and Shinn (*4*). Mackinney (*6*) found the major carotene fraction of leaves to consist of beta-carotene; grass contained only beta-carotene with no detectable amounts of alpha-carotene present.

The average number of Sherman-Munsell units per microgram of carotene was less for two samples of yellow corn than it was in alfalfa or peanut hay. This may be accounted for by the fact that the actual pigments in yellow corn consist in large part of cryptoxanthin (40 to 70 percent, according to the writers' results), which has a lower vitamin-A potency than beta-carotene. However, there may be appreciable errors in the results with yellow corn because too few samples were used. More data are being obtained for this feed. The carotene in one sample of Johnson-grass hay also had less vitamin A potency than the average for carotene in peanut or alfalfa hay.

It is possible that at higher levels of feed there may be a poorer utilization of carotene. Ahmad (*1*) found a variation in the utilization of ingested carotene due to imperfect absorption from the gut. This

and other factors such as differences in digestibility due to the nature of the plant tissue and differences in the kind of carotene found may cause variations in the utilization of plant carotenes at higher levels. This work is being continued.

SUMMARY

Seven samples of alfalfa products containing from 7.3 to 63.5 micrograms of carotene had a vitamin A potency of from 13 to 77 Sherman-Munsell units (15.6 to 92.4 international units) per gram, with an average value of 1.4 units (1.6 international units) per microgram of carotene. Five samples of peanut hay ranging from 5.6 to 26.5 micrograms of carotene per gram had an average value of 1.3 Sherman-Munsell units (1.5 international units) per microgram of carotene.

Two samples of yellow corn containing 1.7 and 2.6 micrograms of carotene per gram had an average value of 1.2 Sherman-Munsell units (1.4 international units) per microgram of carotene, while one sample of Johnson grass hay containing 9.3 micrograms of carotene per gram had a vitamin A potency of 1.1 Sherman-Munsell units (1.3 international units) per microgram of carotene.

One microgram of carotene in the international standard had a value of 1.4 Sherman-Munsell units, and 1 microgram of purified carotene had the same value. Carotene in alfalfa products and peanut hay had practically the same vitamin-A potency, expressed in Sherman-Munsell units, as the carotene in the international standard and in purified carotene dissolved in oil.

LITERATURE CITED

(1) Ahmad, B.
1931. The fate of carotene after absorption in the animal organism. Biochem. Jour. 25: 1195–1204, illus.

(2) Fraps, G. S., and Treichler, R.
1933. Vitamin A content of foods and feeds. Tex. Agr. Expt. Sta. Bull. 477, 34 pp.

(3) Guilbert, H. R.
1934. Determination of carotene as a means of estimating the vitamin A value of forage. Indus. and Engin. Chem., Analyt. Ed. 6: 452–454.

(4) Hartman, A. M., Kane, E. A., and Shinn, L. A.
1934. Does the carotene in alfalfa hay account completely for its vitamin A activity? Jour. Biol. Chem. (Amer. Soc. Biol. Chem. Proc. 28) 105: xxxvi.

(5) Kuhn, R., and Grundmann, C.
1933. Über kryptoxanthin, ein xanthophyll der formel $C_{40}H_{56}O$. (Über das vitamin des wachstums, v. mitteil.). Ber. Deut. Chem. Gesell. 66 (Bd. 2, Abt. B): 1746–1750, illus.

(6) Mackinney, G.
1935. Leaf carotene. Jour. Biol. Chem. 111: 75–84.

(7) Moore, T.
1930. Vitamin A and carotene. V. The absence of the liver oil vitamin A from carotene. VI. The conversion of carotene to vitamin A in vivo. Biochem. Jour. 24: 692–702, illus.

(8) Morgan, A. F., and Madsen, E. O.
1933. A comparison of apricots and their carotene equivalent as sources of vitamin A. Jour. Nutrition 6: 83–93, illus.

U. S. GOVERNMENT PRINTING OFFICE: 1936